2002

Lake food webs: Biological and chemical perspectives

Craig P. Stafford
The University of Montana

LAKE FOOD WEBS: BIOLOGICAL AND CHEMICAL PERSPECTIVES

by

Craig P. Stafford

B.S., University of Michigan, Ann Arbor, 1991

M.S., University of Maine, Orono, 1994

Presented in partial fulfillment of the requirements

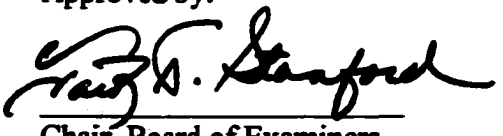
for the degree of

Doctor of Philosophy

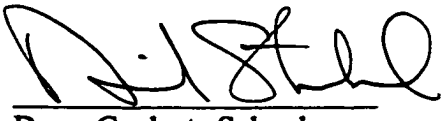
The University of Montana

2002

Approved by:



Chair, Board of Examiners



Dean, Graduate School

5-28-02

Date

ABSTRACT

Stafford, Craig P., Ph.D., May 2002 Division of Biological Sciences

Lake Food Webs: Biological and Chemical Perspectives

Advisor: Jack A. Stanford JAS.

I observed no relationship between fish growth rate and mercury concentration in a lake trout (*Salvelinus namaycush*) population and conditional support for an inverse relationship in a smallmouth bass (*Micropterus dolomieu*) population in two Maine lakes. A bioenergetics model indicated that mercury concentration was more responsive to dietary mercury intake than to growth rate. Because fish obtain mercury primarily from their diet, these findings suggest that food web structure and individual diet preferences may influence fish mercury contamination.

I found mercury levels in lake trout, lake whitefish (*Coregonus clupeaformis*), and their major diet items increased with site depth in Flathead Lake, Montana. These findings imply that individual fish have some long term preferences in foraging depth, and strongly suggest that habitat use can affect fish contamination.

I found that $\delta^{13}C$ and $\delta^{15}N$ of lake trout, lake whitefish, and their major prey items were generally related to site depth in Flathead Lake. I used $\delta^{13}C$ as a proxy for average foraging depth in the fishes and found a significant relationship between $\delta^{13}C$ and mercury contamination. I used $\delta^{15}N$ as a measure of fish trophic position and found a positive relationship between mercury contamination and $\delta^{15}N$. However, the interpretation of the nitrogen results is obscured by correlations among $\delta^{15}N$, depth, and mercury.

$\delta^{15}N$ signature of *Mysis relicta* from the isotope study suggests they obtain substantial quantities of pelagic nitrogen during their diurnal migration to feed on pelagic zooplankton. Using a multi lake study I found lakes with Mysis had a lower proportion of cladocerans (the preferred prey of Mysis) in the vicinity of the thermocline. These finding illustrate how Mysis predation in the water column shunts zooplankton production from the water column to the bottom, allowing deep water fishes to proliferate.

Using otoliths I compared lake trout growth pre and post Mysis in Flathead Lake. Widths of annual increments 1 to 3 were similar, but generally declined in increments 4 to 10 post Mysis. These results suggest that increased growth was not the mechanism by which lake trout populations expanded post Mysis. However, the energetic shuttle of zooplankton production by Mysis foraging may have increased deep water foraging opportunities for small lake trout.

ACKNOWLEDGMENTS

I thank my advisor Jack Stanford and the graduate committee of Andy Sheldon, Mark Lorang, Ric Hauer, and Johnnie Moore for reviewing this work. I acknowledge the help of numerous people who helped me in the field, most of whom generously volunteered their time. Levia Jones and Angelika Buscha assisted me during many lake collections and in the lab as well. Brooke Baldauf, Jake Chaffin, Samantha Chilcote, Bonnie Ellis, Mary Harner, Vicki Ludden, Brandon Jackson, David Pepin, David Phillips, and Andi Shockley also helped with lake sampling. I thank Mark Deleray, Jon Graham, Dave Smith, and Mark Trudel for comments on various manuscripts. I am very grateful to David Beauchamp for providing diet data on Flathead Lake fishes, and to Barry Hansen for lengths of angler caught lake trout in Flathead Lake. I thank the Maine Department of Inland Fisheries and Wildlife for collecting and aging the Maine fishes, as well as the Salish-Kootenai Tribal Fisheries Program and the Montana Department of Fish, Wildlife and Parks for capturing Flathead Lake fishes. Funding for aspects of various projects was provided by the Flathead Lake Biological Station, Salish-Kootenai Tribal Fisheries Program, Montana Department of Fish, Wildlife and Parks, U. S. Environmental Protection Agency, and Maine Department of Environmental Protection. I thank the Flathead Lake Biological Station for financially supporting me through my research assistant position, and the Division of Biological Sciences for supporting me through teaching assistantships.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

TABLE Page

CHAPTER 1

INTRODUCTION

Craig P. Stafford

Viewing organisms as food web components has provided a productive context for examining patterns in community ecology. By examining the flow of energy through food webs (Elton 1927; Lindeman 1942) together with inter specific interactions such as predator-prey relationships and competition (Hairston et al. 1960; Brooks and Dodson 1965, Paine 1966, Hurlbert et al. 1972) ecologists have been able to address many fundamental questions in community ecology. Increasingly, however, ecologists are reversing this logic and using the flow of energy and materials through food webs to gain insight into the ecology of the component species. A prominent approach is to follow the flow of materials through food webs using chemical tracers such as stable isotopes.

In this research I used stable isotopes and mercury tracers combined with traditional organismal investigations to examine lentic food webs. The research was focused on Flathead Lake, Montana, where the introduction of the opossum shrimp *Mysis relicta* has reconfigured the food web. Mysis feeds voraciously on large zooplankton in the water column, but resides on the bottom during the day. The feeding habits of Mysis shuttles macro zooplankton production from the pelagic zone to the profundal, thereby increasing the abundance of deep water fishes. In Flathead Lake, the establishment of

Mysis coincided with a dramatic increase in benthic species, in particular lake trout (*Salvelinus namaycush*) and lake whitefish (*Coregonus clupeaformis*).

The research begins in Maine with Chapter 2 where I utilized two relatively large data sets of mercury in fish. I examine the influence of fish diet and growth rate on mercury contamination in smallmouth bass (*Micropterus dolomieu*) and lake trout. The goal of this research was to elucidate if fish diet preference (and thus food web structure) or fish growth is more important in determining mercury contamination. I continued the mercury work on Flathead Lake in Chapter 3 where more detailed information was collected on the life history and diet of lake trout and lake whitefish. The objective of this work was to evaluate the importance of life history features and foraging habitat on mercury contamination, and to use the mercury patterns to investigate the habitat use by the fishes. In Chapter 4 I further explored the role of individual fish foraging habitat on mercury contamination with an isotope tracer study in lake trout, lake whitefish, and their major diet items in Flathead Lake. I quantified foraging depth and trophic position using isotopes to investigate if these factors are related to mercury contamination at the individual fish scale. To better understand how Mysis shuttle energy to these benthic fishes, I conducted a multi lake study in Chapter 5 examining the impact of Mysis predation on the distribution of zooplankton in northwest Montana lakes. The impact of this energetic pump was subsequently examined in Chapter 6 where I examine impact Mysis introduction had on the growth of lake trout in Flathead Lake.

REFERENCES

Brooks J. L. & Dodson S. I. (1965) Predation, body size, and composition of plankton. *Science* 150:28-35.

Elton, C. 1927. Animal Ecology. Sidgwick & Jackson, LTD., London, England.

Hairston, N. G., F. E. Smith, and L. B. Slobodkin. 1960. Community structure, population control and competition. *The American Naturalist* 94:421-425.

Hurlbert, S. H., J Zedler, and D. Fairbanks. 1972. Ecosystem alteration by mosquitofish (*Gambusia affinis*) predation. *Science* 175:639-641.

Lindeman, R. 1942. The trophic-dynamic aspect of ecology. *Ecology* 23:399-418.

Paine, R. T. 1966. Food web complexity and species diversity. *The American Naturalist* 100:65-75.

CHAPTER 2

MERCURY CONTAMINATION AND GROWTH RATE IN TWO PISCIVORE POPULATIONS

Craig P. Stafford and Terry A. Haines

ABSTRACT

We found no relationship between fish growth rate and mercury concentration in a lake trout population and conditional support for an inverse relationship in a smallmouth bass population. A bioenergetics model indicated mercury concentration was more responsive to dietary mercury intake than to growth rate. When biodilution is evident, it may bias contaminant versus fish size relationships.

INTRODUCTION

Persistent contaminant levels within a fish population vary considerably, even when fish size is taken into account [1-3]. Hypotheses to explain this intra-population variability include differences in individual fish physiology, location, diet and growth rate. Growth rate has the potential to influence persistent contaminant levels in fish via biodilution [2-5]. Bioenergetics models predict that mercury concentration is inversely related to fish growth rate [5,6], but to our knowledge, few empirical investigations have tested these predictions. The objective of the current investigation was to examine the empirical relationship between mercury levels and growth rate in two piscivore

populations. To explore these findings, we used a bioenergetics model to examine the relative sensitivity of growth rate and dietary variation in influencing fish mercury concentrations. We conclude by discussing the potential of growth effects to bias contaminant versus fish size relationships.

METHODS

Lake trout (*Salvelinus namaycush*) from Moosehead Lake (44° 35' N, 69° 40' W) and smallmouth bass (*Micropterus dolomieu*) from Carlton Pond (44° 42' N, 69° 16' W) Maine, USA, were sampled in the summer of 1994. Fish were captured with gill and trap nets and aged by counting scale annuli. Smallmouth bass ranged from two to 20, and lake trout one to 12 years of age. Approximately 10 g of dorsal epaxial muscle was taken from the left side of each fish, placed in a clean glass vial, and frozen. Sixty-three lake trout and 57 smallmouth bass were analyzed for total mercury at the Orono Field Station of the United States Geological Survey (Orono, Maine, USA). We did not use two smallmouth bass because of an exceptionally high mercury value in a five year old fish (1205 ng/g wet weight) and a suspected measuring error in another fish.

Total mercury levels were determined using an atomic fluorescence spectrophotometer. A sub-sample of 1.5 g of wet tissue was placed in a pressure containment vessel and microwave digested in a 1:1 (v/v) mixture of concentrated nitric acid and hydrogen peroxide. Quality assurance included standard reference material (dogfish muscle certified reference material, Institute for National Measurement Standards, Ottawa, ON, Canada), standard reference material spikes, and replicates of digested samples.

We investigated the relationship between mercury levels and growth rate using 3- to 12-year-old lake trout (n = 59) and 4- to 12-year-old smallmouth bass (n = 41). We did not include the younger fish because of the small sample size, and we did not include the older smallmouth bass because of their slow growth rate. We used partial regressions to determine if mercury concentration was related to growth rate. We regressed log mercury (Hg [ng/g wet weight] versus total length (TL [mm]), as well as log age (years) versus TL to obtain the residuals. We used the residuals of the first regression to obtain a size-normalized mercury concentration index and the negative residuals of the second regression to obtain a growth rate index. We regressed the mercury concentration residuals versus the growth rate residuals to examine the relationship between mercury contamination and growth rate after accounting for length.

We used Fish Bioenergetics 3.0a for Windows (University of Wisconsin Sea Grant Institute, Madison, Wisconsin, USA) to investigate the relative sensitivity of growth rate versus dietary mercury intake in influencing fish methyl mercury concentrations. We used a gross assimilation efficiency model with allometric clearance rate scaling of the form

$$dB_{pred}/dt = C_{prey} * X_{prey} * X_{ae} - (M_{pred}^{\zeta} * B_{pred} * K_{cl}) \qquad (1)$$

where d is change, B_{pred} is the predator contaminant burden, C_{prey} is the mass of prey consumed per unit time, X_{prey} is the prey contaminant concentration, X_{ae} is the gross assimilation efficiency, M_{pred} is the predator mass, ζ is the allometric constant, and K_{cl} is the elimination constant. This model assumes that all of the contaminant inputs come from diet. We performed a 120 day 8°C isothermal simulation for a 1000 g lake trout using the physiological parameter defaults. Mercury concentrations were calculated at

two growth regimes (50 and 75 g), each with two X_{prey} (180 and 240 ng/g wet weight). We assumed a prey energy density = 6000 joules/g wet weight, $X_{ae} = 0.80$ [7], $\zeta = -0.20$ [8], starting $B_{pred} = 5 \times 10^5$ ng, and $K_{cl} = 0.0050$ $g^{-\zeta}/d$ [8]. The model output was expressed as concentration.

RESULTS

The measured mercury levels in the standard reference material (certified range, 4.38 to 4.90 μg/g dry weight) were 4.51 μg/g dry weight (standard deviation [SD] = 0.34 μg/g; n = 12) in the lake trout analysis and 4.68 μg/g dry weight (SD = 0.57 μg/g; n = 20) in the smallmouth bass analysis. Matrix spikes averaged 106.9% recovery (SD = 4.5%; n = 6) in the lake trout analysis and 92.9% recovery (SD = 9.3%; n = 4) in the smallmouth bass analysis. The relative percent difference for replicates was less than 6% for all samples (lake trout, n = 8; smallmouth bass, n = 17).

The relationship between log Hg and TL, for the lake trout, was log Hg = 0.00145TL + 2.10 ($p < 0.001$, $R^2 = 0.627$), and, for the smallmouth bass, was log Hg = 0.00310TL + 1.57 ($p < 0.001$, $R^2 = 0.608$). The relationship between log age and TL, for the lake trout, was log age = 0.00213TL - 0.150 ($p < 0.001$, $R^2 = 0.883$), and, for the smallmouth bass, was log age = 0.00274TL - 0.187 ($p < 0.001$, $R^2 = 0.764$). We found no relationship between the residuals of log Hg versus TL and the negative residuals of log age versus TL for the lake trout ($p = 0.430$, $R^2 = 0.011$) (Fig. 1). Lake trout scales can underestimate the age of older fish [9], so we re-calculated the residual regression using fish less than 400 mm TL and also found no significant relationship ($p = 0.292$, $R^2 = 0.037$). No relationship existed between log Hg versus TL and the negative residuals of

log age versus TL for the smallmouth bass ($p = 0.057$, $R^2 = 0.089$). However, the shortest smallmouth bass was identified as an outlier for the log Hg versus TL (studentized residual = -3.26) and the residual regressions (studentized residual = 3.78). The re-calculated regressions without this outlier were log Hg = 0.00268TL + 1.74 ($p < 0.001$, $R^2 = 0.590$), and log age = 0.00286TL - 0.234 ($p < 0.001$, $R^2 = 0.773$). Using these regressions, a significant relationship was found between the residuals of log Hg versus TL and the negative residuals of log age versus TL: $Y = -0.740X - 0.000$ ($p = 0.001$, $R^2 = 0.265$) (Fig. 2).

Increasing the growth rate 50% slightly reduced the predator mercury concentration under both prey contamination regimes. A 50% increase in the prey mercury level substantially increased the predator mercury concentration under both growth regimes (Table 1).

DISCUSSION

Our empirical findings provide no support for a relationship between mercury concentration and growth rate in the lake trout, and conditional evidence of an inverse relationship in the smallmouth bass. The greater growth variation in the smallmouth bass sample (lower R^2 in the length versus age regression) and the more easily read scales may have increased the probability of detecting growth effects.

Several field studies have shown an inverse relationship between fish mercury and growth rate when growth rate variation is high. Rask et al. [10] reported that a 10-fold increase in growth rate (measured as weight) of perch (*Perca fluviatilis*) after a fish kill was associated with a 50% decline in mercury levels. Braune [11] found a negative

relationship between fish growth rate and mercury concentration in age one- and two-year-old-Atlantic herring (*Clupea harengus harengus*), but not for the three- to five-year-old fish. The relative growth rate variation was highest in the young fish, potentially increasing the possibility of detecting growth effects. Doyon et al. [12] reported that dwarf lake whitefish (*Coregonus clupeaformis*) bioaccumulated mercury more rapidly than normal individuals in the same reservoir, despite similar methyl mercury levels in dietary items.

Field studies have presented unclear evidence for biodilution of mercury when growth rate variation is moderate or low. Verta [1] investigated changes in fish mercury associated with the removal of half the fish biomass in a lake. Growth rates in age three- to six-year-old pike (*Esox lucius*) approximately doubled after removal, whereas mercury concentrations fell. The author maintained the reduced pike mercury levels may have been caused by increased growth rate. However, whether the mercury concentrations in pike food items remained constant is unclear, because mercury levels in the forage fish generally declined after fish removal. Further, similar mercury declines in the burbot (*Lota lota*) and small roach (*Rutilus rutilus*) occurred despite similar growth rates before and after fish removal. Munn and Short [13] reported that mercury levels in composites of 33- to 41-cm TL walleye (*Stizostedion vitreum*) were negatively related to condition factor, which the authors attributed to biodilution. However, this study confounded site differences in fish contamination and condition factor by combining multiple composites from each of three sites into one analysis. This is particularly problematic, because fish from the least contaminated site had the highest condition factor.

Our modeling results provide insight into the contrasting findings on mercury biodilution. Predator mercury concentrations were relatively unresponsive to changes in growth but responded strongly to changes in prey mercury levels, as has been documented previously [6]. If individual fish specialize in certain prey species, sizes of a given species, or foraging areas, this variability in diet could result in differential contaminant uptake. As a result, any biodilution easily could be obscured if individual fish have dietary preferences, particularly if growth rate variation is low or if growth rate and mercury intake covary. Recent isotope research on northern pike (*Esox lucius*) strongly suggests these fish have individual feeding preferences [14], lending credence to the idea that intrapopulation dietary variation occurs. Differences in individual fish physiology also could obscure biodilution.

When losses of a diet-obtained contaminant are minimal, the relative importance of contaminant biodilution should increase. Thus, we expect biodilution will be more pronounced for strongly biomagnified compounds. Sijm et al. [15] reported biodilution in guppies (*Poecilia reticulata*) increased in more persistent polychlorinated biphenyl congeners. Borgman and Whittle [5] reported a lower predator-to-prey biomagnification factor for mercury (~5) than polychlorinated biphenyl and dichlorodiphenyldichloroethylene (~10 for both) in adult lake trout (five to eight years old). Correspondingly, their modeling results indicated biodilution was more important for dichlorodiphenyldichloroethylene and polychlorinated biphenyls than for mercury.

Biodilution has the potential to bias contaminant versus fish size relationships when collection methods are size selective. Size-selective methods can capture fish with

unrepresentative growth rates, potentially biasing contaminant versus fish size relationships if biodilution is substantial.

A subtle regression bias can occur when growth rate influences contaminant levels. Slow-growing fish spend more time at small sizes, and conversely, rapid growth decreases this time. The result is that slow-growing fish may be captured more often at small sizes and fast-growing fish at large sizes. This can bias the size-adjusted contaminant levels if slow-growing fish with high size-specific contaminant levels are captured more often at smaller sizes and fast-growing fish with low size-specific contaminant levels are captured more often at larger sizes. This bias will decrease the slope of the length versus contaminant regression line, resulting in an overestimate of the size adjusted contaminant burdens in large fish and, conversely, an underestimate in slow growing fish. This is analogous to body versus otolith size back calculations. Slow-growing fish have otoliths that are larger at a given body size, resulting in back calculated lengths that can underestimate the actual size of slow-growing fish [16].

Despite the potential of biodilution to bias contaminant versus fish size relationships, many investigators do not evaluate growth effects or even report fish ages. Further, comparisons of contaminant levels among sites routinely use length as a covariate to account for differences in size [17]. When this technique is used to compare environmental contamination between collection areas, it is implicit that fish growth rate does not affect contaminant burdens, or growth rate does not vary among areas. Neither of these assumptions can be tested, however, if the fish are not aged.

In summary, our empirical results, modeling, and literature review suggest that biodilution of mercury can occur. However, diet variability could easily obscure growth

dilution of mercury in field studies. When contaminant biodilution is substantial, the potential exists to bias contaminant versus size relationships. We recommend aging fish in contaminant investigations whenever feasible so that growth biases can be evaluated, particularly if working with highly persistent chemicals.

Acknowledgement-The U.S. Environmental Protection Agency funded the mercury analysis through the Maine Department of Environmental Protection. The Flathead Lake Biological Station supported C. Stafford during manuscript preparation. Fish were captured and aged by the Maine Department of Inland Fisheries and Wildlife. R. Perry performed the mercury analysis. M. Trudel, D. Smith, and J. Graham provided manuscript comments.

REFERENCES

1. Verta M. 1990. Changes in fish mercury concentrations in an intensively fished lake. *Can J Fish Aquat Sci* 47:1888-1897.
2. Hammar J, Larsson P, Klavins M. 1993. Accumulation of persistent pollutants in normal and dwarfed arctic char (*Salvelinus alpinus* sp. complex). *Can J Fish Aquat Sci* 50:2574-2580.
3. Madenjian CP, Carpenter SR, Rand PS. 1994. Why are the PCB concentrations of salmonine individuals from the same lake so highly variable? *Can J Fish Aquat Sci* 51:800-807.
4. Thomann RV. 1989. Bioaccumulation model of organic chemical distribution in aquatic food chains. *Environ. Sci Technol* 23:699-707.
5. Borgmann U, Whittle DM. 1992. Bioenergetics and PCB, DDE, and mercury dynamics in Lake Ontario lake trout (*Salvelinus namaycush*): A model based on surveillance data. *Can J Fish Aquat Sci* 49:1086-1096.
6. Harris RC, Bodaly RA. 1998. Temperature, growth and dietary effects on fish mercury dynamics in two Ontario lakes. *Biogeochemistry* 40:175-187.
7. Norstrom RJ, McKinnon AE, de Freitas ASW. 1976. A bioenergetics-based model for pollutant accumulation by fish. Simulation of PCB and methylmercury residue levels in Ottawa River yellow perch (*Perca flavescens*). *J Fish Res Board Can* 33:248-267.
8. Trudel M, Rasmussen JB. 1997. Modeling the elimination of mercury by fish. *Environ Sci Technol* 31:1716-1722.

9. Burnham-Curtis MK, Bronte CR. 1996. Otoliths reveal a diverse age structure for humper lake trout in Lake Superior. *Trans Am Fish Soc* 125:844-851.

10. Rask M, Järvinen M, Kuoppamäki K, Pöysä H. 1996. Limnological responses to the collapse of the perch population in a small lake. *Ann Zool Fenn* 33:517-524.

11. Braune BM. 1987. Mercury accumulation in relation to size and age of Atlantic herring (*Clupea harengus harengus*) from the southwestern Bay of Fundy, Canada. *Arch Environ Contam Toxicol* 16:311-320.

12. Doyon JF, Schetagne R, Verdon R. 1998. Different mercury bioaccumulation rates between sympatric populations of dwarf and normal lake whitefish (*Coregonus clupeaformis*) in the La Grande complex watershed, James Bay, Quebec. *Biogeochemistry* 40:203-216.

13. Munn MD, Short TM. 1997. Spatial heterogeneity of mercury bioaccumulation by walleye in Franklin D. Roosevelt Lake and the Upper Columbia River, Washington. *Trans Am Fish Soc* 126:477-487.

14. Beaudoin CP, Tonn WM, Prepas EE, Wassenaar LI. 1999. Individual specialization and trophic adaptability of northern pike (*Esox lucius*): an isotope and dietary analysis. *Oecologia* 120:386-396.

15. Sijm DTHM, Seinen W, Opperhuizen A. 1992. Life-cycle biomagnification study in fish. *Environ Sci Technol* 26:2162-2174.

16. Campana SE. 1990. How reliable are growth back-calculations based on otoliths. *Can J Fish Aquat Sci* 47:2219-2227.

17. Somers KM, Jackson DA. 1993. Adjusting mercury concentration for fish-size covariation: a multivariate alternative to bivariate regression. *Can J Fish Aquat Sci* 50:2388-2396.

Table 1. Modeled lake trout mercury levels (ng/g wet weight) under two prey mercury concentrations (ng/g wet weight) and two growth rates (g/120 days).

	growth=50	growth=75
prey=180	509	505
prey=240	542	540

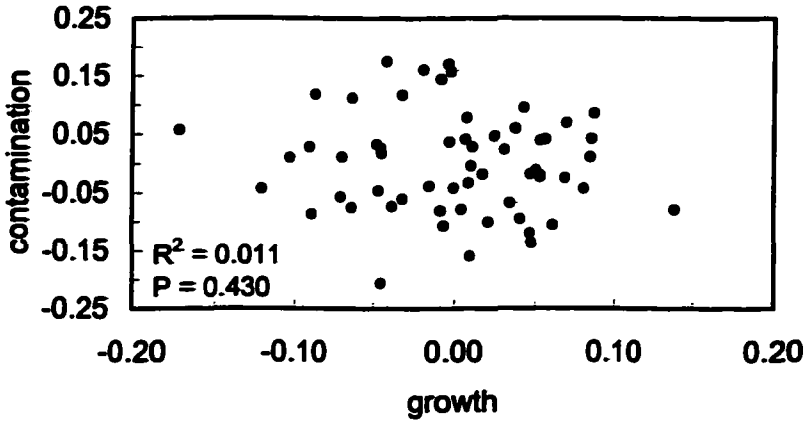


Fig. 1. Residuals of log Hg versus length (contamination) versus negative residuals of log age versus length (growth) for the 3- to 12-year-old lake trout.

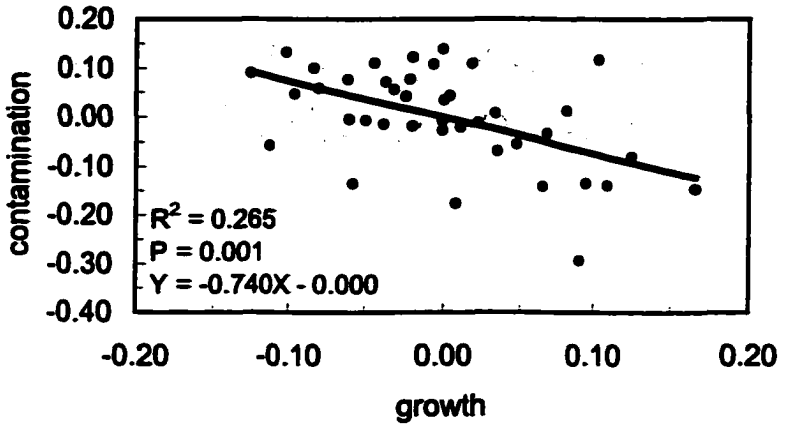


Fig. 2. Residuals of log Hg versus length (contamination) versus residuals of log age versus length (growth) with the outlier removed for the 4- to 12-year-old smallmouth bass.

CHAPTER 3

MERCURY IN THE FOOD WEB OF FLATHEAD LAKE, MONTANA

Craig P. Stafford, Barry Hansen, and Jack A. Stanford

ABSTRACT

We evaluated the role of life history characteristics and habitat in predicting mercury contamination in two fish species. Mercury levels in lake trout, lake whitefish, and their major diet items were investigated in Flathead Lake, Montana. For both fish species, mercury increased with size and age, and showed a negative relationship with growth rate. No gender based differences in mercury levels were observed for either fish species. A positive relationship between mercury concentration and depth was documented for both fish species and their major diet items, suggesting that individual fish have long term habitat preferences. These findings underscore the need to consider biological attributes of organisms when conducting contaminant assessments, and the usefulness of contaminants as food web tracers.

INTRODUCTION

Mercury contamination in fish is a natural occurrence that has been exacerbated by human activities. In non industrialized areas, the atmosphere appears to be the primary source of labile mercury entering watersheds, although geologic sources also can be important especially in regions with mercury rich formations (Shilts and Coker 1995).

Atmospheric mercury has local (Harrison and Klaverkamp 1990), regional (Nater and Grigal 1992), and global (Slemr and Langer 1992) components. Mason et al. (1994) concluded that two-thirds of the global mercury flux through the atmosphere is related to anthropogenic sources. Coal combustion and refuse incineration are the largest anthropogenic sources of mercury to the atmosphere (Nriagu and Pacyna 1988).

Although mercury inputs from the atmosphere are dominated by inorganic mercury (Fitzgerald et al. 1991), most of the mercury in fish is methyl mercury (Bloom 1992). Methyl mercury is produced in water bodies and their watersheds, although some methyl mercury is present in precipitation (Fitzgerald et al. 1991, Watras et al. 1995). Sulfate reducing bacteria appear to be the primary producer of methyl mercury in aquatic ecosystems (Gilmour and Henry 1991). Methyl mercury biomagnifies through the food chain, and large predatory fish often reach levels that exceed public health guidelines.

Studies of mercury contamination in fish have generally emphasized the role of environmental features, particularly water chemistry, in predicting mercury contamination in fish. Recently, however, increasing attention has been given to the role of biological attributes such as life history patterns (Riget et al. 2000), food web structure (Cabana and Rasmussen 1994), and habitat (Monteiro et al. 1996) in determining fish mercury levels. For this assessment, we documented mercury levels in lake trout (*Salvelinus namaycush*) and lake whitefish (*Coregonus clupeaformis*) from Flathead Lake, Montana in relation to life history characteristics (size, age, sex, and growth rate), habitat (depth of capture), as well as mercury levels in the major diet items of both fishes. We were particularly interested in any insights the mercury results could provide into the

habitat preferences of these fishes. We also compared mercury levels in Flathead Lake fishes to other non-industrialized North American lakes.

METHODS

Study Site

Flathead Lake is located in the Upper Columbia Basin of northwest Montana (47° 52' N, 114° 04' W) and is the largest natural lake (482 km^2) in the Pacific Northwest (Spencer et al. 1991). The lake is oligotrophic with an average depth of 52 m and a mean water retention time of < 3 years (Potter and Stanford 1974, Spencer et al. 1991). The watershed is predominantly forested and approximately two-thirds lies within protected wild lands (Stanford and Hauer 1992). Following the establishment of the non-native freshwater shrimp *Mysis relicta* in 1981 (Spencer et al. 1991), populations of non-native lake trout and lake whitefish expanded dramatically in Flathead Lake (Stafford et al. in press). The lake supports an important sport fishery as well as a subsistence fishery for members of the Confederated Salish-Kootenai Tribes. The catch is dominated by lake trout with some harvest of yellow perch (*Perca flavescens*), lake whitefish, and limited harvest of other species (Evarts et al. 1994).

Sample Collection and Preparation

Fish were captured using sinking experimental gill nets (bar mesh sizes from 19 to 51 mm) from 25 April 2000 to 04 May 2000 on the south half of Flathead Lake as part of a large scale fishery monitoring program. Thirty lake trout and 26 lake whitefish were used in this assessment. Fish were transported on ice and total length (TL in mm), mass (g), sex, maturity and the average net depth (m) were recorded. Lake trout ranged from

336 to 987 mm TL and lake whitefish TL ranged from 143 to 630 mm TL. Scales (lake whitefish) and otoliths (lake whitefish and lake trout) were collected for aging, and the fish were subsequently wrapped in aluminum foil and frozen. Later, the fish were partially thawed, the right fillet was removed to the posterior margin of the dorsal fin and a skinless piece of muscle tissue was taken dorsal of the body cavity. This tissue was placed in a plastic bag, frozen, and shipped to Brooks Rand Ltd. (Seattle, Washington) on dry ice for homogenization and total mercury analysis.

Benthic invertebrates were collected 25-26 October 2000 using a benthic sled at six sites on the south half of Flathead Lake. Representative samples of Mysis and dipterans (almost exclusively chironomids) were sorted on the day of collection in an acid washed plastic bin, and invertebrates were briefly rinsed in deionized water. Invertebrate samples were placed in an acid washed glass jar and inverted to drain the excess water. Samples were stored frozen and shipped on dry ice to Brooks Rand Ltd. for homogenization and methyl mercury analysis. Moisture content was determined from a separate sample at 85% for Mysis and 86% for dipterans.

Mercury Analysis

Fish were analyzed for total mercury and invertebrates were analyzed for methyl mercury using cold vapor atomic fluorescence spectrophotometry. Total mercury served as a surrogate for methyl mercury in the fish analysis because of methyl mercury's dominance in fish (Bloom 1992), whereas invertebrates were analyzed for methyl mercury due to the substantial quantities of inorganic mercury often present in invertebrates (Tremblay 1999). Fish samples were digested in 7:3 mixture (by volume) of nitric and sulfuric acids, followed by the addition of bromine monochloride. Mercury

was volatilized to Hg^0 using stannous chloride, purged, and captured onto a gold trap. The trap was subsequently heated, releasing the mercury to the fluorescence detector. Invertebrate tissue was digested in a mixture of 25% potassium hydroxide and 75% methanol (by volume) and was subsequently brought to a known volume with methanol. Mercury species were ethylated and captured in a Tenax trap. The ethyl mercury derivatives were thermally desorbed and transferred to a gas chromotography column. The methyl mercury was decomposed to Hg^0 and detected by fluorescence. Quality assurance included duplicate samples, dogfish muscle certified reference material (DORM-2, Institute for National Measurement Standards, Ottawa, Ontario), certified reference material spikes, and blanks. All samples were blank corrected. Mercury results are presented in ng/g wet weight unless otherwise noted.

For the fish analysis, the DORM-2 (certified value = 4640 ng/g dry weight total mercury) results averaged 4264 ng/g dry weight (range = 3929 to 4757, s.d. = 309, n = 6), DORM-2 spikes averaged 97.2% recovery (range = 82.3 to 109.1, s.d. = 9.8, n = 6), blanks averaged 0.78 ng/g (range = 0.14 to 3.26, s.d. = 1.22, n = 6), and duplicates averaged 6.0% relative percent difference (range = 0.5 to 20.5, s.d. = 7.7, n = 6). The methyl mercury analysis was conducted twice for the Mysis and once for the dipterans (because of insufficient mass for a second analysis). The DORM-2 (certified value = 4470 ng/g dry weight methyl mercury) averaged 3440 ng/g dry weight (range = 2768-4405, s.d. = 653, n = 6), DORM-2 spikes averaged 113% recovery (range = 90.6 to 135, s.d. = 31.5, n = 2), blanks averaged 1.24 ng/g (range = 0 to 2.54, s.d. = 1.44, n = 4), and duplicates averaged 3.84 relative percent differences (s.d. = 1.34, range = 2.55 to 5.23, n = 3) and 21.8 relative standard deviation (s.d. = 7.71, range = 16.3 to 27.2, n = 2). One

dipteran sample was analyzed in a separate analysis (DORM-2 = 4470 ng/g dry weight methyl mercury, blanks = 0.00 and 2.37 ng/g). Mercury levels in two of the dipteran samples were below the detection limit of 3.34 ng/g. We approximated these samples at one half detection limit for the data analysis. Methyl mercury levels in the certified reference material were biased low and the mercury levels in the invertebrates were less than five times the minimum detection limit of the method. Thus the invertebrate methyl mercury values should be considered approximate.

Fish Aging

Dry otoliths were cleaned in a 5% solution of household bleach and rinsed in deionized water. Otoliths were cleared in a mixture of 90% glycerin and 10% water (by volume). Otoliths were read whole in water against a black background with a dissecting scope. Impressions of lake whitefish scales were made in acetate using a heated hydraulic press and viewed with a microfiche reader. Ages were assigned to lake whitefish based on simultaneous inspection of scales and otoliths. Fish were assigned ages by the number of one year periods since hatching (rounded to the nearest year). Lake trout ages ranged from 5 to 31 years and lake whitefish from 1 to 9 years.

Fish Mercury versus Other Studies

We compared mercury levels in the Flathead Lake lake trout and lake whitefish with other studies of non-industrialized lakes. Reservoir studies were avoided because impoundment generally increases fish mercury levels (Bodaly et al. 1984), and we did not use studies that indicated the presence of geological formations high in mercury. Log mercury concentration (ng/g) versus TL (mm) from Flathead Lake lake trout were compared with lake trout from Cayuga Lake, New York, U.S.A. (Gutenmann et al. 1992),

Moosehead Lake, Maine, U.S.A. (Stafford and Haines 2001) and 96 lakes in Ontario, Canada (Cabana et al. 1994). Cabana et al. (1994) reported the lake trout mercury versus mass relationship depended on food chain length, with contamination lowest in lakes with no Mysis and no forage fish (Class 1), intermediate with forage fishes but no Mysis (Class 2), and highest with Mysis and forage fish (Class 3). Thus, we presented the mercury versus TL relationship for each of the three different food chain configurations. Total lengths for Cabana et al. (1994) were estimated from mass using fish from the current study: $mm = 62.9\ g^{0.304}$ ($R^2 = 0.98$, $P < 0.01$). For lake whitefish, we made comparisons with four lakes in Northwest Territories, Canada (Stephens 1995), 86 lakes in Quebec, Canada (Schetagne and Verdon 1999), as well as Lake Serigny and Lac Rond-de-Poêle northern Quebec, Canada (Trudel et al. 2001). These studies present arithmetic mean mercury levels, thus we used a mercury versus length exponential curve for lake whitefish from Flathead Lake. Lake whitefish fork lengths from Stephens (1995) were converted to total lengths using: TL = 1.12 fork length (Carlander 1969). Total lengths for Trudel et al. (2001) were estimated from mass using the fish from this study: $mm = 59.5\ g^{0.307}$.

Fish Diet Data

Lake trout and lake whitefish diet data were determined from fish collected throughout Flathead Lake in experimental gill nets (bar mesh sizes from 13 to 76 mm) from May to June 1998, October to November 1998, and March to June 1999. Stomach contents were preserved in 10% formalin (by volume), and the masses (nearest 0.1 g) of diet items from each non-empty stomach were calculated as a percentage. These

percentages were subsequently averaged for lake trout between 200-500 and 501-1000 mm TL and lake whitefish between 200-370 and 371-650 mm TL.

RESULTS

Mercury in Fish

Mercury levels increased with fish mass, length and age for both the lake trout and the lake whitefish (Table 1). We removed one 814 mm TL lake trout from the analysis because it appeared to be a high outlier and had high leverage on the regressions. We created a size independent index of growth rate using the residuals of the mass versus age for the lake trout: log g = 1.92 log years + 1.07 ($R^2 = 0.85$, $P < 0.01$); and for the lake whitefish: log g = 1.92 log years + 1.45 ($R^2 = 0.90$, $P < 0.01$). Using t-tests, we detected no differences in the growth rate between sexes for the lake trout ($P = 0.88$) or lake whitefish ($P = 0.60$). To evaluate the relationship between growth rate and contamination, we created a multiple regression model using growth rate and mass to predict mercury concentration. We found a negative relationship between mercury and growth rate for both fish species with mass in the model (Table 2)

To evaluate any sex based differences in mercury versus size relationships, we created a multiple regression model of log mercury versus TL, sex, and the TL by sex interaction for both fishes. For the lake trout, no interaction existed ($P = 0.89$), so this term was dropped. No differences were observed between males and females in the abbreviated model ($P = 0.43$). No interaction existed in the lake whitefish full model ($P = 0.96$), so the interaction term was dropped. No difference existed between males and females in the abbreviated model ($P = 0.83$). Some caution is warranted in the lake

whitefish gender comparison, however, as females had a higher average TL (females = 448, males = 329).

To examine the relationship between fish contamination and depth of capture, the residuals of log Hg versus length (Table 1) were used as size independent contamination indices. Contamination increased with depth for the lake trout: mercury index = 0.00173 m - 0.0648 ($R^2 = 0.23$, $P = 0.01$, Figure 1); and for the lake whitefish: mercury index = 0.00305 m - 0.0697 ($R^2 = 0.16$, $P = 0.04$, Figure 2). No relationship was detected between growth rate index and depth for the lake trout ($R^2 = 0.07$, $P = 0.18$) or lake whitefish ($R^2 = 0.10$, $P = 0.11$). Some caution is warranted with the lake whitefish depth findings as relatively few fish were caught from deep water.

On a size normalized basis, mercury levels in lake trout from Flathead Lake were less contaminated than Moosehead Lake, Cayuga Lake, and Ontario lakes that contain Mysis and pelagic forage fish. Similar lake trout mercury levels existed between Flathead Lake and Ontario lakes which contain pelagic forage fish, but no Mysis, and lower levels existed in Ontario lakes with no Mysis or pelagic forage fish (Figure 3). Mercury levels in Flathead Lake lake whitefish were lower than Lake Serigny and 86 Quebec lakes. Lake whitefish mercury levels in four Northwest Territories lakes were lower than Flathead Lake, while levels in Lac Rond-de-Poêle were similar (Figure 4).

Invertebrate Mercury

The mean methyl mercury concentration for dipterans was 8.8 ng/g and for Mysis was 9.6 ng/g. No relationship between mercury and depth was detected for either dipterans ($R^2 = 0.46$, $P = 0.13$) or Mysis ($R^2 = 0.35$, $P = 0.22$) alone. To examine if the sampled invertebrates as a whole were more contaminated in relation to depth of

collection, we created a multiple regression model of both species with mercury as the dependent variable and depth, species, and the interaction as predictors. No interaction ($P = 0.28$) was detected in the full model, so this term was dropped. No species effect ($P = 0.79$) was detected in the abbreviated model, so this term was also dropped. A positive relationship between pooled invertebrate mercury and depth existed in the reduced model: $ng/g = 0.145\ m + 2.53$ ($R^2 = 0.35$, $P = 0.04$, Figure 5).

Fish Diet Data

Smaller lake trout fed primarily on Mysis, while larger lake trout had a more varied diet with substantial consumption of lake whitefish and Mysis (Table 3). Diet shifts with size were less evident in the lake whitefish. Lake whitefish diet was dominated by dipterans and also included substantial amounts of Mysis and bivalves (Table 4).

DISCUSSION

Mercury levels in lake trout and lake whitefish increased with fish size and age, which has been observed for many fish species. In general as fish grow, they consume bigger, more contaminated diet items. Further, larger predatory fish tend to have higher predator to prey biomagnification ratios (Borgmann and Whittle 1992). This appears to be the case for both lake trout and the lake whitefish from Flathead Lake. The 200-500 mm lake trout had approximately twice the mercury of the Mysis that comprised 75% of their diet, while the 500-1000 mm fish had about five times more mercury than the Mysis and small whitefish that comprised 40% of their diet. The lake whitefish diet did not

change much with size. However, larger individuals were still more contaminated also suggesting larger fish have higher biomagnification ratios.

A negative relationship existed between mercury concentration and growth rate for both lake trout and lake whitefish. Fast growing fish are expected to have lower persistent contaminant levels because of biodilution (the dilution of a chemical within an organism by the addition of new tissue) (Thomann 1989). However, biodilution may not always be evident in field studies of mercury contamination, especially when growth variation is low or when differences in individual fish diet, physiology, etc. affect mercury levels and obscure biodilution (Stafford and Haines 2001).

Many collection methods will capture fish with growth rates that are not representative of the population being sampled. Therefore, when growth rates are an important determinant of fish contaminant levels, the collection method can influence the contaminant results. In this study, the fish were captured with gill nets that may select fast growing young fish because they are more mobile. Indeed, one and two year old lake trout captured in gill nets in another study on Flathead Lake tended to be fast growers (Stafford et al. in press). The youngest lake trout used in this study was five years old, so it is unlikely that growth effects substantially biased the lake trout mercury results. However, the lake whitefish sample contained fish as young as age one. Thus, it is possible that the mercury levels reported here for young lake whitefish were a slight underestimate of the actual population.

For both fish species, age was the best predictor (highest R^2) of mercury concentration, particularly in the lake whitefish sample. This may occur because age

integrates two factors that were predictors of fish mercury concentration in this analysis: fish size and growth rate.

No growth differences between sexes were observed for either fish species, which has been reported previously for lake trout (Allen-Gil et al. 1997). The greater energetic requirements of females and the low mercury losses to eggs [see Lange et al. (1994) for mercury concentrations in ovaries] could lead to higher mercury levels in females. However, higher mercury levels have been found in males (Olson 1976, Phillips et al. 1980, Lange et al. 1993) and females (Nicoletto and Hendricks 1988, Monteiro et al. 1991), suggesting differences largely are due to idiosyncratic sexual variation in factors such as diet, physiology, growth, and habitat use.

The increase in fish and invertebrate mercury with depth could be caused by higher concentrations of methyl mercury in deeper water. Although we lack data for Flathead Lake, increasing methyl mercury concentration with depth has been observed in several other lakes (Bloom et al. 1991, Meuleman et al. 1995, Rask and Verta 1995). Methyl mercury production is greatest in shallow sediments (Ramlal et al. 1993), however downward transport of particulate bound methyl mercury and subsequent release (Hurley et al. 1991) and photodegradation of methyl mercury in surface waters (Sellers et al. 1996) appear to be particularly important in creating the observed profiles. Mercury concentration in the sediments may also influence benthic invertebrate mercury levels, particularly sediment dwelling taxa such as dipterans.

The ecology of the biota also may be important in influencing mercury versus depth relationships. Mysis in Flathead Lake have a one year life cycle (Chess and Stanford 1998), and the average size of Mysis has been observed to increase with depth

in Flathead Lake (Jack Stanford, unpublished data) and in other systems (Morgan and Threlkeld 1982). In a Mysis population with a two year life cycle, the younger (smaller) age class had lower mercury levels (Sandlund et al. 1987). If the larger Mysis from Flathead Lake are more contaminated, this could contribute to the observed invertebrate mercury increase with depth.

The higher mercury levels of lake whitefish and lake trout from deeper sites provide insight into the ecology of these fishes. Fish obtain most of their mercury through diet (Hall et al. 1997). Because lake whitefish, lake trout, and their primary diet items are more contaminated with depth of capture, it appears that individuals of both fish species have long term depth preferences. These findings illustrate how contaminants can be used as tracers to gain insight into the ecology of mobile species (also see Bayne et al. 2002), and the need to consider individual fish habitat preferences when sampling for environmental contaminants.

Persistent contaminant levels within fish populations often are highly variable even after accounting for body size. Modeling work by Madenjian et al. (1993, 1994) supports the idea that variation in dietary contaminant intake of individual fish contributes substantially to contaminant variation within fish populations. Our research suggests that habitat preferences of individual fish can contribute to variation in dietary contaminant intake when habitat and contamination are related thereby increasing contaminant variation within fish populations.

On a size normalized basis, muscle tissue mercury in fish from Flathead Lake appear typical relative to other published North American studies of non industrialized lakes, with some suggestion the lake trout were below the geometric average. These

findings generally are in agreement with Watras et al. (1995) who compared waterborne mercury in remote Glacier National Park, Montana, U.S.A. lakes (approximately 100 km north of Flathead Lake) to remote Wisconsin and New York, U.S.A. lakes and found lower total and methyl mercury concentrations in the northwest Montana lakes. High mercury levels (>1000 ng/g) do exist in the lake trout, however, because of the large size this species attains in Flathead Lake. Some caution is warranted in the lake trout comparison as all the cited studies are from northeastern North America where fish mercury levels generally are high (Stafford and Haines 1997). In the native range of lake trout, mercury levels have been shown to be higher in the presence of Mysis, apparently due to greater biomagnification through a longer food chain (Sprules and Bowerman 1988, Cabana et al. 1994, Cabana and Rasmussen 1994). Compared to Quebec lakes with a similar trophic structure (Class 3, Mysis and pelagic forage fish present), the mercury levels in the Flathead Lake fish are lower. It is possible that the establishment of Mysis increased mercury levels in lake trout from Flathead Lake, but no pre Mysis lake trout mercury data are available for comparison.

Overall these findings illustrate the need to consider life history, habitat preferences, and other biological attributes of the study organisms when conducting contaminant assessments. Specifically, our data show that fish mercury concentrations were related to size, age, growth rate, and habitat. The increase in mercury levels with depth in both fish species and their diet items suggests that individual fish have long term habitat preferences, illustrating the usefulness of contaminants as food web tracers.

Acknowledgment-The Salish-Kootenai Tribal Fisheries Program captured the fish, funded the mercury analyses, and provided diet data in cooperation with David Beauchamp. The Flathead Lake Biological Station provided boat time for invertebrate collection and financial support for Craig Stafford during fish preparation, aging and manuscript writing. Angelika Buscha assisted with invertebrate collection.

REFERENCES

Allen-Gil, S. M., C. P. Gubala, D. H. Landers, B. K. Lasorsa, E. A. Crecelius, and L. R. Curtis. 1997. Heavy metal accumulation in sediment and freshwater fish in U.S. Arctic lakes. *Environmental Toxicology and Chemistry* 16:733-741.

Bayne, D. R., E. Reutebuch, and W. C. Seesock. 2002. Relative motility of fishes in a Southeastern reservoir based on tissue polychlorinated biphenyl residues. *North American Journal of Fisheries Management* 22:122-131.

Bloom, N. S. 1992. On the chemical form of mercury in edible fish and marine invertebrates tissue. *Canadian Journal of Fisheries and Aquatic Sciences* 49:1010-1017.

Bloom, N. S., C. J. Watras, and J. P. Hurley. 1991. Impact of acidification on the methylmercury cycle of remote seepage lakes. *Water, Air, and Soil Pollution* 56:477-491.

Bodaly, R. A., R. E. Hecky, and R. J. P. Fudge. 1984. Increases in fish mercury levels in lakes flooded by the Churchill River diversion, northern Manitoba. *Canadian Journal of Fisheries and Aquatic Sciences* 41:682-691.

Borgmann, U., and D. M. Whittle. 1992. Bioenergetics and PCB, DDE, and mercury dynamics in Lake Ontario lake trout (*Salvelinus namaycush*): a model based on surveillance data. *Canadian Journal of Fisheries and Aquatic Sciences* 49:1086-1096.

Cabana, G., and J. B. Rasmussen. 1994. Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. *Nature* 372:255-257.

Cabana, G., A. Tremblay, J. Kalff, and J. B. Rasmussen. 1994. Pelagic food chain structure in Ontario lakes: a determinant of mercury levels in lake trout. *Canadian Journal of Fisheries and Aquatic Sciences* 51:381-389.

Carlander, K. D. 1969. Handbook of Freshwater Fishery Biology Volume One. Iowa State University Press, Ames, Iowa.

Chess, D. W., and J. A. Stanford. 1998. Comparative energetics and life cycle of the opossum shrimp (*Mysis relicta*) in native and non-native environments. *Freshwater Biology* 40:783-794.

Evarts, L., B. Hansen, and J. DosSantos. 1994. Flathead lake angler survey. DOE/BP-60479-1. Bonneville Power Administration, Portland, Oregon.

Fitzgerald, W. F., R. P. Mason, and G. M. Vandal. 1991. Atmospheric cycling and air-water exchange of mercury over mid-continental lacustrine regions. *Water, Air, and Soil Pollution* 56:745-767.

Gilmour, C. C., and E. A. Henry. 1991. Mercury methylation in aquatic systems affected by acid deposition. *Environmental Pollution* 71:131-169.

Gutenmann, W. H., J. G. Ebel, H. T. Kuntz, K. S. Yourstone, and D. J. Lisk. 1992. Residues of p,p'-DDE and mercury in lake trout as a function of age. *Archives of Environmental Contamination and Toxicology* 22:452-455.

Hall, B. D., R. A. Bodaly, R. J. P. Fudge, J. W. M. Rudd, and D. M. Rosenberg. 1997. Food as the dominant pathway of methylmercury uptake by fish. *Water, Air, and Soil Pollution* 100:12-24.

Harrison, S. E., and J. F. Klaverkamp. 1990. Metal contamination in liver and muscle of northern pike (*Esox lucius*) and white sucker (*Catostomus commersoni*) and in

sediments from lakes near the smelter at Flin Flon, Manitoba. *Environmental Toxicology and Chemistry* 9:941-956.

Hurley, J. P., C. J. Watras, and N. S. Bloom. 1991. Mercury cycling in a northern Wisconsin seepage lake: the role of particulate matter in vertical transport. *Water, Air, and Soil Pollution* 56:543-552.

Lange, T. R., H. E. Royals, and L. L. Connor. 1993. Influence of water chemistry on mercury concentration in largemouth bass from Florida lakes. *Transactions of the American Fisheries Society* 122:74-84.

Lange, T. R., H. E. Royals, and L. L. Connor. 1994. Mercury accumulation in largemouth bass (*Micropterus salmoides*) in a Florida lake. *Archives of Environmental Contamination and Toxicology* 27:466-471.

Madenjian, C. P., S. R. Carpenter, G. W. Eck, and M. A. Miller. 1993. Accumulation of PCBs by lake trout (*Salvelinus namaycush*): an individual-based model approach. *Canadian Journal of Fisheries and Aquatic Sciences* 50:97-109.

Madenjian, C. P., S. R. Carpenter, and P. S. Rand. 1994. Why are the PCB concentrations of salmonine individuals from the same lake so highly variable? *Canadian Journal of Fisheries and Aquatic Sciences* 51:800-807.

Mason, R. P., W. F. Fitzgerald, and F. M. M. Morel. 1994. The biogeochemical cycling of elemental mercury: anthropogenic influences. *Geochimica et Cosmochimica Acta* 58:3191-3198.

Meuleman, C., M. Leermakers, and W. Baeyens. 1995. Mercury speciation in Lake Baikal. *Water, Air, and Soil Pollution* 80:539-551.

Monteiro, L. R., V. Costa, R. W. Furness, and R. S. Santos. 1996. Mercury concentrations in prey fish indicate enhanced bioaccumulation in mesopelagic environments. *Marine Ecology Progress Series* 141:21-25.

Monteiro, L. R., E. J. Isidro, and H. D. Lopes. 1991. Mercury content in relation to sex, size, age and growth in two scorpionfish (*Helicolenus dactylopterus* and *Pontinus kuhlii*) from Azorean waters. *Water, Air, and Soil Pollution* 56:359-367.

Morgan, M. D., and S. T. Threlkeld. 1982. Size dependent horizontal migration of *Mysis relicta*. *Hydrobiologia* 93:63-68.

Nater, E. A., and D. F. Grigal. 1992. Regional trends in mercury distribution across the Great Lakes states, north central USA. *Nature* 355:139-141.

Nicoletto, P. F., and A. C. Hendricks. 1988. Sexual differences in accumulation of mercury in four species of centrarchid fishes. *Canadian Journal of Zoology* 66:944-949.

Nriagu, J. O., and J. M. Pacyna. 1988. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *Nature* 333:134-139.

Olson, M. 1976. Mercury levels as a function of fish size and age in northern pike. *Ambio* 5:73-77.

Phillips, G. R., T. E. Lenhart, and R. W. Gregory. 1980. Relation between trophic position and mercury accumulation among fishes from the Tongue River Reservoir, Montana. *Environmental Research* 22:73-80.

Potter, D. S., and J. A. Stanford. 1975. Influences on the plankton communities of oligotrophic Flathead Lake. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie* 19:1790-1797.

Ramlal, P. S., C. A. Kelly, J. W. M. Rudd, and A. Furutani. 1993. Sites of methyl mercury production in remote Canadian Shield lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 50:972-979.

Rask, M., and M. Verta. 1995. Concentrations and amounts of methylmercury in water and fish in the limed and acid basins of a small lake. *Water, Air, and Soil Pollution* 80:577-580.

Riget, F., G. Asmund, and P. Aastrup. 2000. Mercury in Arctic char (*Salvelinus alpinus*) populations from Greenland. *The Science of the Total Environment* 245:161-172.

Sandlund, O. T., G. Kjellberg, and G. Norheim. 1987. Kvikksølv i fisk og evertebrater i Mjøsa. *Fauna* 40:10-15.

Schetagne, R., and R. Verdon. 1999. Mercury in fish of natural lakes of northern Québec. Pages 115-130, *In* M. Lucotte, R. Schetagne, N. Therien, C. Langlois, and A. Tremblay (editors) Mercury in the Biogeochemical Cycle: Natural Environments and Hydroelectric Reservoirs of Northern Québec (Canada). Springer-Verlag, Berlin, Germany.

Sellers, P., C. A. Kelly, J. W. M. Rudd, and A. R. MacHutchon. 1996. Photodegradation of methylmercury in lakes. *Nature* 380:694-697.

Shilts, W. W., and W. B. Coker. 1995. Mercury anomalies in lake water and in commercially harvested fish, Kaminak Lake area, District of Keewatin, Canada. *Water, Air, and Soil Pollution* 80:881-884.

Slemr, F., and E. Langer. 1992. Increase in global atmospheric concentrations of mercury inferred from measurements over the Atlantic Ocean. *Nature* 355:434-437.

Spencer, C.N., B. R. McClelland, and J. A. Stanford. 1991. Shrimp stocking, salmon collapse, and eagle displacement. *BioScience* 41:14-21.

Sprules, W.G., and J.E. Bowerman. 1988. Omnivory and food chain length in zooplankton food webs. *Ecology* 69:418-426.

Stanford, J. A., and F. R. Hauer. 1992. Mitigating the impacts of stream and lake regulation in the Flathead River catchment, Montana, USA: an ecosystem perspective. *Aquatic Conservation: Marine and Freshwater Ecosystems* 2:35-63.

Stafford, C. P., and T. A. Haines. 1997. Mercury concentrations in Maine sport fishes. *Transactions of the American Fisheries Society* 126:144-152.

Stafford, C. P., and T. A. Haines. 2001. Mercury contamination and growth rate in two piscivore populations. *Environmental Toxicology and Chemistry* 20:2099-2101.

Stafford, C. P., J. A. Stanford, F. R. Hauer, and E. B. Brothers. In press. Changes in lake trout growth associated with *Mysis relicta* establishment: a retrospective analysis using otoliths. *Transactions of the American Fisheries Society.*

Stephens, G. R. 1995. Mercury concentrations in fish in a remote Canadian Arctic lake. *Water, Air, and Soil Pollution* 80:633-636.

Thomann, R. V. 1989. Bioaccumulation model of organic chemical distribution in aquatic food chains. *Environmental Science and Technology* 23:699-707.

Tremblay, A. 1999. Bioaccumulation of mercury and methylmercury in invertebrates from natural boreal lakes. Pages 89-113, *In* M. Lucotte, R. Schetagne, N. Therien, C. Langlois, and A. Tremblay (editors) Mercury in the Biogeochemical Cycle: Natural Environments and Hydroelectric Reservoirs of Northern Québec (Canada). Springer-Verlag, Berlin, Germany.

Trudel, M., A. Tremblay, R. Schetagne, and J. B. Rasmussen. 2001. Why are dwarf fish so small? An energetic analysis of polymorphism in lake whitefish (*Coregonus clupeaformis*). *Canadian Journal of Fisheries and Aquatic Sciences* 58: 394-405.

Watras, C. J., K. A. Morrison, and N. S. Bloom. 1995. Mercury in remote Rocky Mountain lakes of Glacier National Park, Montana, in comparison with other temperate North American regions. *Canadian Journal of Fisheries and Aquatic Sciences* 52:1220-1228.

TABLE 1. Relationships between fish mercury versus age, mass, and total length.

Species	Equation	R^2	P
Lake trout	log ng/g = 0.0362 years + 2.06	0.87	<0.01
Lake trout	log ng/g = 0.000101 g + 2.33	0.84	<0.01
Lake trout	log ng/g = 0.00129 mm + 1.78	0.85	<0.01
Lake trout	$ng/g = 59.9e^{0.00298\ mm}$	0.84	<0.01
Lake trout[1]	$ng/g = 56.3e^{0.00313\ mm}$	0.79	<0.01
Lake whitefish	log ng/g = 0.0804 years + 1.73	0.62	<0.01
Lake whitefish	log ng/g = 0.000261 g + 1.94	0.38	<0.01
Lake whitefish	log ng/g = 0.00126 mm + 1.60	0.51	<0.01
Lake whitefish	$ng/g = 39.8e^{0.00291\ mm}$	0.51	<0.01

[1]outlier included

TABLE 2. Multiple regression models of fish mercury versus mass and growth rate.

Species	Dependent	Predictor	Coefficient	P	Model R^2
Lake trout	log hg	g	0.000109	<0.01	0.87
		growth	-0.309	0.02	
		constant	2.31	<0.01	
Lake whitefish	log hg	g	0.000311	<0.01	0.50
		growth	-0.490	0.03	
		constant	1.91	<0.01	

TABLE 3. Total length (mm), percent weight of diet items, and sample size for lake trout captured 1998-1999 in Flathead Lake.

TL (mm)	*M. relicta*	Lake whitefish	Other	N
200-500	74.8	4.4	20.8	149
501-1000	18.4	21.7	59.8	177

TABLE 4. Total length (mm), percent weight of diet items, and sample size for lake whitefish captured 1998-1999 in Flathead Lake.

TL (mm)	*M. relicta*	Dipteran	Bivalve	Other	N
200-370	8.3	44.4	17.1	30.1	79
371-650	16.5	53.6	15.6	14.2	157

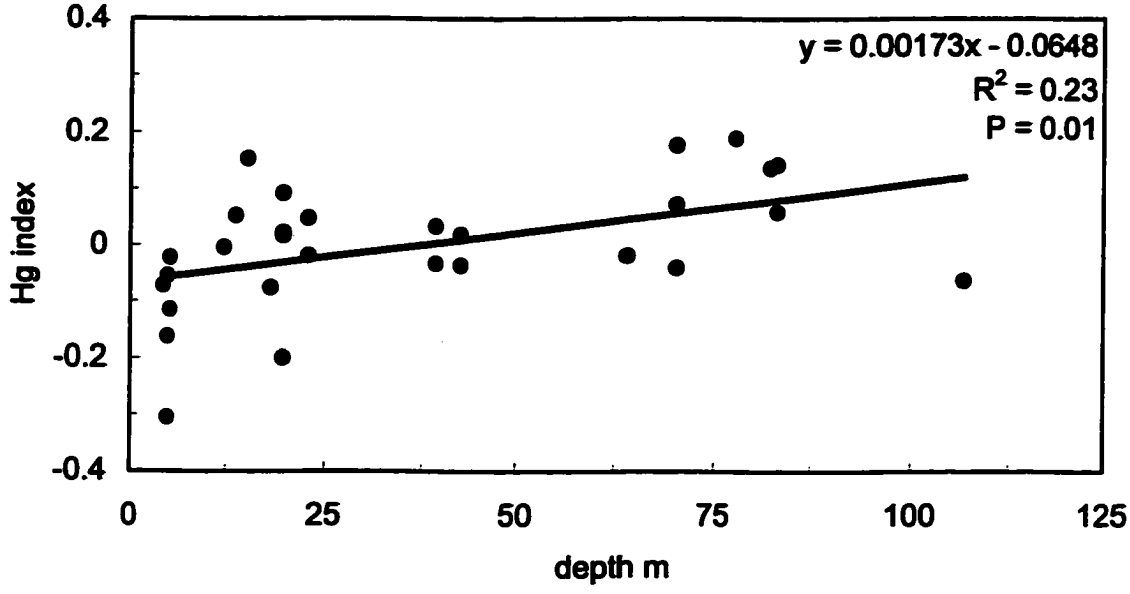


Figure 1. Lake trout length based mercury index versus depth of capture.

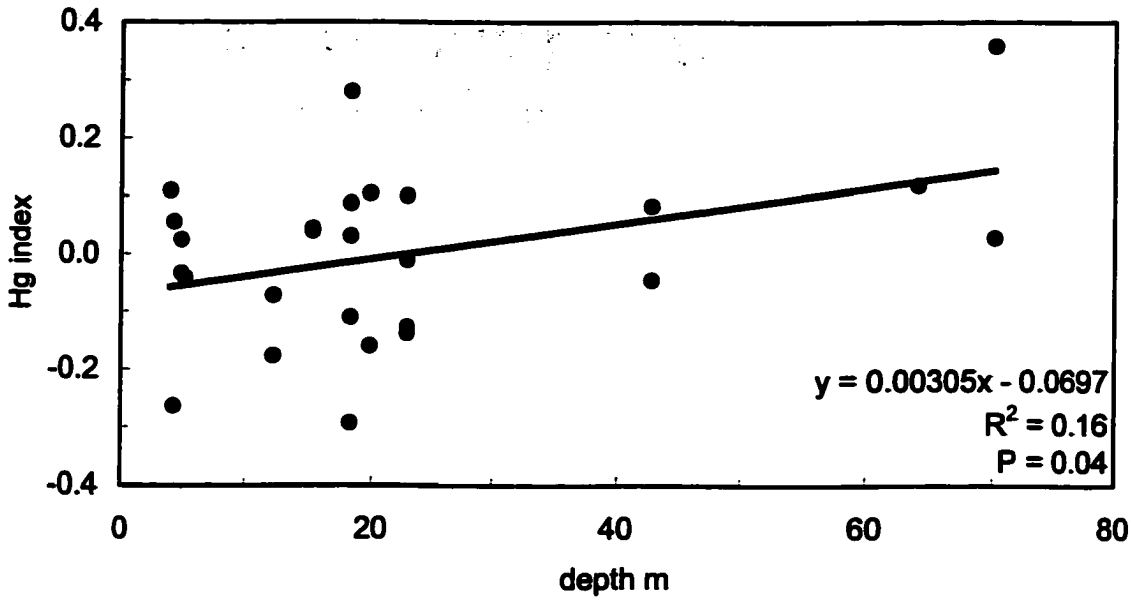


Figure 2. Lake whitefish length based mercury index versus depth of capture.

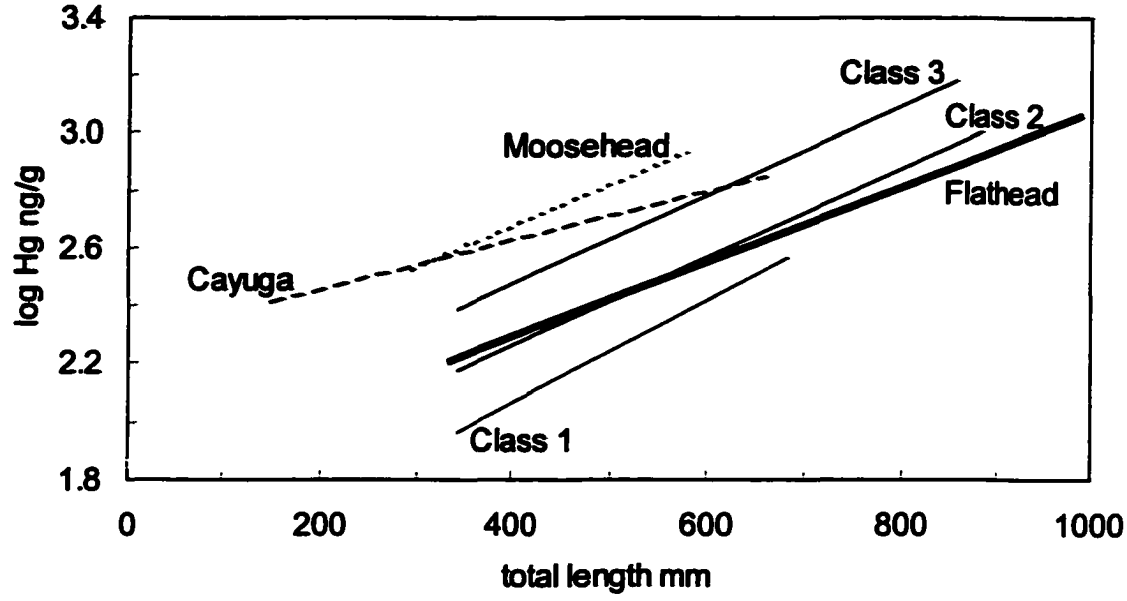


Figure 3. Lake trout mercury versus total length for North American lakes. Flathead = current study; Moosehead = Moosehead Lake, Maine, U.S.A.; Cayuga = Cayuga Lake, New York, U.S.A.; Class 1 = Quebec, Canada lakes with no pelagic forage fish or *M. relicta*; Class 2 = Quebec lakes with pelagic forage fish and no *M. relicta*; Class 3 = Quebec lakes with pelagic forage fish and *M. relicta*.

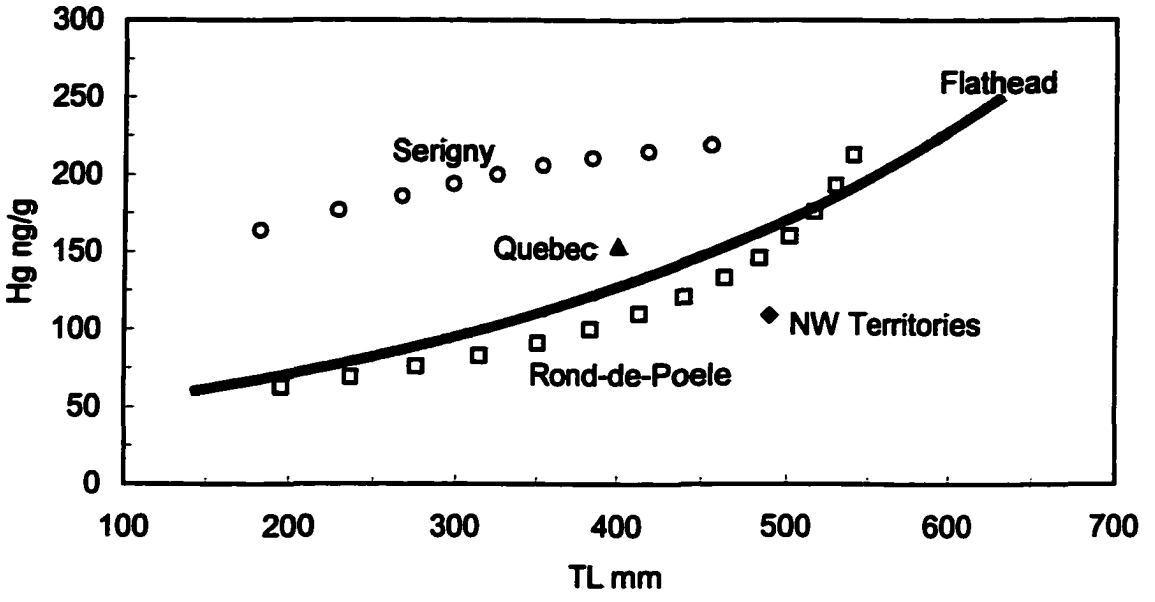


Figure 4. Lake whitefish mercury versus total length for North American lakes. Flathead = current study; Serigny = Lake Serigny, Northern Quebec Canada; Rond-de-Poele = Lac Rond-de-Poêle, Northern Quebec Canada; Quebec = 86 lakes, Quebec Canada; NW Territories = four lakes, Northwest Territories Canada.

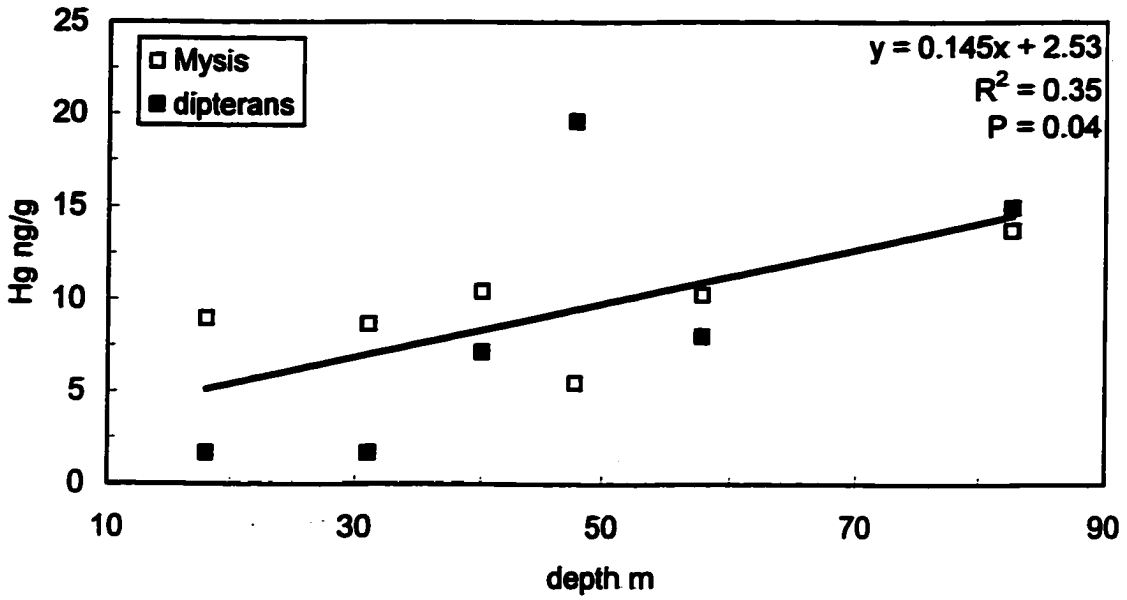


Figure 5. Pooled *M. relicta* and dipteran methyl mercury versus depth of capture.

CHAPTER 4

TROPHIC POSITION, HABITAT USE, AND MERCURY IN FISH: INSIGHTS FROM STABLE ISOTOPES

Craig P. Stafford and Jack A. Stanford

ABSTRACT

We measured $\delta^{13}C$ and $\delta^{15}N$ in lake trout (*Salvelinus namaycush*), lake whitefish (*Coregonus clupeaformis*), and their major prey items to evaluate habitat use (foraging depth) and trophic position of individual fish in Flathead Lake, Montana, U.S.A. We subsequently applied the quantification of foraging depth and trophic position based on the isotope results to investigate mercury contamination in individual fish of both species. $\delta^{13}C$ of lake trout and lake whitefish declined with site depth, and $\delta^{13}C$ of their major prey items declined or showed no relationship with site depth. The general correspondence between the isotope ratios in the fish and their diet item findings strongly suggests that individual fish have long term preferences in foraging depth. We used $\delta^{13}C$ as a proxy for average foraging depth in the fishes and found a negative relationship between $\delta^{13}C$ and mercury contamination in a pooled sample. We used $\delta^{15}N$ as a measure of trophic position and found a negative relationship between mercury contamination and $\delta^{15}N$ in a pooled sample, but interpretation of these findings was hindered by correlations among mercury, $\delta^{15}N$, and depth.

INTRODUCTION

Studies using chemical tracers increasingly are being used in ecological investigations. A prominent approach is to use naturally occurring variation in stable isotope ratios to trace the flow of elements through food webs. By tracing elemental flow it is possible to gain insight into the structure and function of food webs, and the ecology of the component species as reflected by their consumption patterns.

The carbon and nitrogen in the soft tissue of animals are obtained through diet. An animal's isotopic signature for diet obtained elements reflects the isotope ratio of assimilated foods and subsequent losses. This signature will be weighted towards recent consumption, especially when organisms exhibit high metabolic (Frazer et al. 1997) and relative growth rates (Fry and Arnold 1982, Hesslein et al. 1993). Thus smaller organisms generally reflect the isotope intake over a shorter time period. The isotope ratios of ^{13}C to ^{12}C (relative to the PeeDee limestone reference, as $\delta^{13}C$) and of ^{15}N to ^{14}N (relative to the air reference, as $\delta^{15}N$) can be used to examine the diet preference provided that the various food items have distinguishable isotope profiles (Haines and Montague 1979, Estep and Vigg 1985). Further, if variation in diet obtained isotopes is related to habitat it may be possible to characterize habitat usage and migration as well (Fry 1981, Hesslein et al. 1991).

Studies of individual fish habitat and diet preferences traditionally have been difficult in large, deep lakes. Researchers investigating these preferences in a fish's natural setting required multiple captures or repeated observations of the same fish which is impractical in large, deep lakes. Recently the use of sonar tags has allowed for the examination of habitat use in deep waters, however this approach is expensive and time

consuming. An alternative approach is to examine diet and habitat use of individual fish with chemical tracers such as stable isotopes.

In lakes $\delta^{13}C$ and $\delta^{15}N$ of primary producers varies spatially, and these differences can be transmitted through the food web provided that consumers have some habitat fidelity. Variation in the isotope ratios of primary producers can result from both differential fractionation (Degens et al. 1968; Hecky and Hesslein 1995) and varying isotope ratios in the dissolved pools (Oana and Deevey 1960; Rau 1978). The $\delta^{13}C$ of invertebrates declines from littoral to pelagic to profundal while $\delta^{15}N$ shows the opposite trend, reflecting trends in the isotope ratios of the primary producers on which they feed (Vander Zanden and Rasmussen 1999). The isotope ratio variation with habitat in the primary consumers should provide a useful signal for investigating the diet and habitat preferences of higher consumers, provided that any changes that occur through the food web are taken into account.

In food webs $\delta^{13}C$ and $\delta^{15}N$ exhibit distinct properties. The ratio of ^{13}C to ^{12}C changes little through freshwater food webs (France and Peters 1997) making $\delta^{13}C$ particularly suited for tracing carbon sources in lakes. In contrast, a progressive increase of the $\delta^{15}N$ with trophic level has been observed a variety of food webs (Minagawa and Wada 1984; Cabana and Rasmussen 1994) making $\delta^{15}N$ especially useful in studies of food web length. In fish $\delta^{15}N$ has been used as a measure of trophic position in studies of biomagnified contaminants, often relative to a fixed lower trophic level (Cabana and Rasmussen 1994, Vander Zanden and Rasmussen 1996). A chemical measure of trophic position is useful in toxicological studies because fish obtain biomagnified contaminants primarily through diet (Rasmussen et al. 1990, Hall et al. 1997), and fish feeding on

longer food webs tend to be more contaminated (Akielaszek and Haines 1981, Rasmussen et al. 1990, Vander Zanden and Rasmussen 1996).

Several studies have shown positive relationships between $\delta^{15}N$ and persistent contaminants among numerous taxa (Yoshinaga et al. 1992, Rolff et al. 1993, Kidd et al. 1995, Kidd et al. 1998a, Kidd 1998b, Bowles et al. 2001, but see Campbell et al. 2000). Investigations within fish populations have been conducted, however most focus on the general correspondence between fish contamination and $\delta^{15}N$ without any attempt to evaluate the potentially covarying influence age (but see Kiriluk et al. 1995), size (but see Bowles et al. 2001), or habitat preference.

Persistent contaminant levels in individual fish within a population often vary substantially even after accounting for fish size (Akielaszek and Haines 1981; Madenjian et al. 1993). Modeling work by Madenjian et al. (1993, 1994) suggests that variation in prey contamination is an important component of intra population contaminant variability, but they provided no direct observations that individual fish have prey preferences. However, multiple recapture studies (Bryan and Larkin 1972; Schindler et al. 1997), laboratory observations (Ehlinger and Wilson 1988), and isotope investigations combined with diet work (Gu et al. 1997, Beaudoin et al. 1999) have provided evidence that individual fish within a population can have diet preferences. Together the modeling and empirical diet preference work support the hypothesis that individual fish diet preference can contribute to intra population contaminant variation.

In the current investigation we used stable isotopes to more fully understand how lake food webs and individual fish foraging habitats interact to influence mercury contamination at the individual fish level. Specifically, we measured carbon and nitrogen

isotopes in lake trout (*Salvelinus namaycush*), lake whitefish (*Coregonus clupeaformis*), and their primary prey items to investigate feeding relationships in the upper trophic levels of a large, oligotrophic lake. We used the isotopes to quantify habitat use and trophic position of individual fish. We subsequently applied our quantification of habitat use and trophic position to examine whether these factors were related to mercury contamination at the individual fish level.

METHODS

Study site

The study was conducted on Flathead Lake which lies in the upper Columbia River Basin of northwest Montana, U.S.A. (47° 52N, 114° 04 W). The lake is large (482 km^2), relatively deep (average depth = 52 meters), oligotrophic, monomictic (except in uncommon years when it freezes), and has a water residence time of less than 3 years (Potter and Stanford 1974; Spencer et al. 1991; Stanford and Ellis in press). The catchment is primarily forested and approximately two thirds lie within protected wild lands (Stanford and Hauer 1992).

The lake underwent a dramatic food web reconfiguration with the establishment of the freshwater shrimp *Mysis relicta* (Spencer et al. 1991; Stanford and Ellis in press). *Mysis relicta* rise from the bottom into the water column at night (Juday and Birge 1927) to feed, primarily on large zooplankton when available (Cooper and Goldman 1980, Spencer et al. 1999). In Flathead Lake juvenile *M. relicta* feed on diatoms, pollen, and unidentified detritus while adults rely heavily on *Daphnia thorata* (Chess and Stanford 1998). The vertical feeding migration of *M. relicta* shuttles macro zooplankton

production from the pelagic zone to the profundal, and *M. relicta* introductions generally have increased the abundance of deep water fishes (Lasenby et al. 1986, Langeland et al. 1991). In Flathead Lake, *M. relicta* establishment coincided with a dramatic increase in the non native lake trout and lake whitefish populations. Small lake trout feed heavily on *M. relicta*, while larger fish rely on lake whitefish, *M. relicta*, and a variety of other items especially littoral fishes. Lake whitefish mostly feed on *M. relicta*, dipterans, and fingernail clams (Chapter 3).

Sample collections

Lake trout and lake whitefish were captured in sinking gill nets from April 25 to May 4, 2000 throughout the south half of Flathead Lake at depths ranging from 4 to 107 m. A piece of dorsal muscle tissue was removed for isotope and mercury analyses. Details of fish collection and aging are in Chapter 3. Lake trout ages ranged from five to 31 years while lake whitefish ranged from one to nine.

Mysis relicta, fingernail clams, and dipterans (almost entirely chironomids) were collected with a benthic sled (500 μm mesh collection bag) at randomly selected sites throughout the south half of the lake at site depths ranging from 15 to 97 m. Invertebrate collections were made on August 15-16, 2000 and March 7, 2001. Invertebrate samples were immediately placed on ice and transported to the lab where they were picked fresh, briefly rinsed in deionized water and frozen. Thawed *M. relicta* were measured from the eye to the tip of the tail, and composites of 15-17 mm and 22-24 mm individuals were made in August and 18-21 mm individuals in March. To examine any size related trends *M. relicta* composites of varying size were prepared from a 55 m deep site in August.

Sample preparation and chemical analysis

Samples were analyzed for $\delta^{15}N$, $\delta^{13}C$, total N and total C using mass spectrometry. All samples were dried at approximately 65°C. The clams were small (2-3 mm) so it was necessary to crush them and pick the meats from the shell after drying. Clam meats were analyzed whole while all other tissue was ground to a fine powder with a mortar and pestle. Approximately 1 mg of dried tissue was sealed in a 5 by 9 mm tin capsule. Fish tissue was analyzed at the Stable Isotope/Soil Biology Laboratory, Institute of Ecology at the University of Georgia, Athens, Georgia U.S.A. Invertebrate tissue was analyzed at the Stable Isotope Research Unit, Department of Crop and Soil Science, Oregon State University, Corvalis, Oregon U.S.A. The fish muscle tissue was also analyzed for total mercury (details in Chapter 3). We assume our invertebrate isotope patterns reflect consumption over weeks or months (Fry and Arnold 1982, Gorokhova and Hansson 1999) while those in the fish represent months or years (Hesslein et al. 1993).

Lipid synthesis decreases $\delta^{13}C$ in animals (Deniro and Epstein 1977, McConnaughey and McRoy 1979) which can complicate the tracing of carbon through food webs. In the current analysis, however, we were interested in using the invertebrate isotope ratios to trace the diet of the fish, thus were not concerned about lipid effects on invertebrate $\delta^{13}C$. The lake whitefish had a very low lipid content (as indicated by a C/N ratio average = 3.3), and we observed no relationship between $\delta^{13}C$ and the C/N ratio ($R^2 = 0.04$, $p = 0.36$). In contrast, the lake trout had a higher lipid content (C/N ratio average = 3.7) and we observed a negative relationship in $\delta^{13}C$ with the C/N ratio: $\delta^{13}C = -1.01$ C/N - 25.8 ($R^2 = 0.18$, $p = 0.02$). Lake trout $\delta^{13}C$ was thus lipid corrected using the procedure of McConnaughey and McRoy (1979).

RESULTS

Invertebrates

A positive relationship between $\delta^{15}N$ and site depth in both seasons, described by an inverse function, was observed for dipterans. The relationship in the summer collection was: $\delta^{15}N = -100.5/m + 9.27$ ($n = 11$, $R^2 = 0.87$, $p < 0.01$), and the winter collection was: $\delta^{15}N = -95.6/m + 11.0$ ($n = 7$, $R^2 = 0.89$, $p = 0.01$) (Fig. 1). To examine if there were seasonal differences in $\delta^{15}N$ we matched samples by site depth and used paired t-tests because of the observed relationship between $\delta^{15}N$ and depth. $\delta^{15}N$ values for the winter collection (mean = 8.58) were higher than the summer collection (mean = 6.51) ($n = 7$ pairs, $t = -4.94$, $p < 0.01$).

Dipteran $\delta^{13}C$ decreased with site depth in the summer, but no detectable relationship existed in the winter. The relationship between summer $\delta^{13}C$ and site depth was described by an inverse function: $\delta^{13}C = 74.4/m - 34.2$ ($n = 7$, $R^2 = 0.45$, $p = 0.02$). In winter no linear relationship ($n = 7$, $R^2 = 0.15$, $p = 0.39$) or inverse relationship ($R^2 = 0.16$, $p = 0.37$) existed (Fig. 2). A t-test detected no differences between the dipteran summer and winter average $\delta^{13}C$ ($n = 18$, $t = -1.00$, $p = 0.33$). The clams collected in summer generally appear have similar isotope trends with site depth as the summer collected dipterans, but too few samples were analyzed for quantitative interpretation (Fig. 1 and Fig. 2).

No relationship existed between *M. relicta* $\delta^{15}N$ and site depth for the summer 15-17 mm ($n = 9$, $R^2 < 0.01$, $p = 0.92$), 22-24 mm ($n = 3$, $R^2 = 0.45$, $p = 0.53$), or winter collections ($n = 7$, $R^2 = 0.19$, $p = 0.33$) (Fig. 3). To examine if there were seasonal

differences in $\delta^{15}N$ we matched samples by site depth and used paired t-tests because of the observed relationship between $\delta^{15}N$ and site depth. $\delta^{15}N$ values for the winter collection (mean = 7.01) were higher than the summer 15-17 mm collection (mean = 5.73) ($n = 7$, $t = -4.89$, $p < 0.01$), but no differences were detected between the winter and summer 22-24 mm (mean = 7.23) collection ($n = 3$ pairs, $t = -1.53$, $p = 0.27$).

No relationship existed between *M. relicta* $\delta^{13}C$ and site depth for the summer 15-17 mm ($n = 9$, $R^2 = 0.11$, $p = 0.39$), summer 22-24 mm ($n = 7$, $R^2 = 0.17$, $p = 0.36$), or winter (d.f. = 5, $R^2 = 0.02$, $p = 0.77$) collections (Fig. 4). We detected a difference in $\delta^{13}C$ between the summer 15-17 mm collection (mean = -31.9) and the winter collection (mean = -33.3) using a t-test ($n = 16$, $t = 2.99$, $p = 0.01$), but no difference between the summer 22-24 mm (mean = -32.8) and the winter collections ($n = 14$, $t = -1.04$, $p = 0.32$).

The *M. relicta* $\delta^{15}N$ versus length regression was described by: $\delta^{15}N = 0.191$ mm +2.87 ($n = 7$, $R^2 = 0.94$, $p < 0.01$). In contrast no relationship existed between $\delta^{13}C$ and length ($n = 7$, $R^2 = 0.08$, $p = 0.53$ (Fig. 5).

Fish

To examine which life history and habitat factors were important predictors of fish $\delta^{15}N$ we created a multiple regression model of TL, site depth, and growth for both species. Growth was quantified using the residuals of the log mass versus log age versus regression for the lake trout: log g = 1.92 log age + 1.07 ($n = 29$ for all lake trout analyses, $R^2 = 0.85$, $p < 0.01$); and for the lake whitefish: log g = 1.90 log age + 1.46 ($n = 25$ for all whitefish analyses, $R^2 = 0.90$, $p < 0.01$). For the lake trout $\delta^{15}N$ full model TL ($p = 0.02$) and site depth ($p = 0.02$) were significant while growth was not significant ($p = 0.73$), thus growth was dropped from the model. The results of this abbreviated model

are presented in Table 1. Both of the independent variables were significant alone as well: $\delta^{15}N = -0.00155$ mm + 12.3 ($R^2 = 0.30$, $p < 0.01$), and $\delta^{15}N = 0.00960$m + 10.9 ($R^2 = 0.28$, $p < 0.01$) (Fig. 6). A model with TL ($p < 0.01$) and growth ($p = 0.34$) did not result in a significant relationship with growth. For the lake whitefish full model all predictors were insignificant: TL ($p = 0.53$), site depth ($p = 0.33$), and growth ($p = 0.16$). Dropping TL did not result in a significant relationship for site depth ($p = 0.33$) or growth ($p = 0.19$), and subsequently dropping site depth did not result in a significant relationship for growth alone ($p = 0.09$). No relationship existed between $\delta^{15}N$ and site depth alone either ($p = 0.16$) (Fig. 7).

We evaluated the predictors of fish $\delta^{13}C$ using the same variables as the nitrogen analysis. In the lake trout full model site depth was significant ($p < 0.01$) while TL ($p = 0.12$) and growth ($p = 0.94$) were not. Growth was dropped from the model and depth remained significant ($p < 0.01$) and TL was not significant ($p = 0.11$). TL was dropped from the model resulting in the relationship $\delta^{13}C = -0.0260$ m - 28.2 ($R^2 = 0.26$, $p < 0.01$) (Fig. 8). No significant effect of growth ($p = 0.68$) was observed with site depth ($p < 0.01$) in the model to predict $\delta^{13}C$, and no relationship existed between $\delta^{13}C$ and growth alone ($p = 0.55$). For the lake whitefish full model growth ($p = 0.60$) and TL ($p = 0.24$) were not significant while site depth ($p = 0.05$) was significant. Growth was dropped from the model, and TL ($p = 0.16$) was not significant and depth ($p = 0.03$) was significant. TL was dropped from the model, resulting in the relationship: $\delta^{13}C = -0.00124$ m - 29.3 ($R^2 = 0.21$, $p = 0.02$) (Fig. 9). No relationship was observed with $\delta^{13}C$ and TL ($p = 0.14$), or $\delta^{13}C$ and growth ($p = 0.15$). No significant effect of growth ($p = 0.38$) was observed with site depth in the model ($p = 0.05$) to predict $\delta^{13}C$.

We detected an inverse relationship between $\delta^{15}N$ and $\delta^{13}C$ in both fish populations. Because the lake trout $\delta^{15}N$ was correlated with TL, a multiple regression model was made using TL and $\delta^{13}C$ to predict $\delta^{15}N$ (Table 2). For the lake whitefish we did not include TL as no relationship between $\delta^{15}N$ and TL was detected earlier. The relationship was described by: $\delta^{15}N = -0.684\delta^{13}C - 12.2$ ($R^2 = 0.22$, $p = 0.02$).

To examine the relationship between habitat usage and mercury contamination we used $\delta^{13}C$ as a proxy for average feeding depth. We based this proxy on the pattern of declining $\delta^{13}C$ with site depth we generally observed in the food web, and that $\delta^{13}C$ shows little change with trophic transfer in freshwater food webs (France and Peters 1997). For the lake trout we created a model of $\delta^{13}C$ and TL to predict log mercury concentration, a weak negative effect of $\delta^{13}C$ ($p = 0.07$) with TL ($p < 0.01$) in the model. Similarly for the lake whitefish we found a weak negative effect of $\delta^{13}C$ ($p = 0.07$) with TL ($p < 0.01$) in the model on log mercury concentration. TL was included in these models because of the significant relationship between log mercury concentration and TL observed in these fish (Chapter 3). To examine if the pooled fish data showed a relationship between $\delta^{13}C$ and mercury contamination we created a model with species, $\delta^{13}C$, and the species by $\delta^{13}C$ interaction to predict size normalized log mercury concentration (calculated as the residual of the log Hg versus TL relationship added to the mean log Hg for each species separately). We detected no significant interaction, so this term was removed from the model ($p = 0.13$). There was a significant relationship between size adjusted log Hg and $\delta^{13}C$ as well as species in the reduced model (Table 3).

We investigated the relationship between fish log mercury concentration and trophic position as indicated by $\delta^{15}N$ using an additive multiple regression approach. We

added TL and then site depth to the models because of positive relationships with these variables and fish mercury were detected in Chapter 3. For the lake trout no relationship between log mercury concentration existed with $\delta^{15}N$ ($p = 0.09$) with TL ($p < 0.01$) in the model. For the lake whitefish no relationship existed between log mercury concentration and $\delta^{15}N$ ($p = 0.11$) with TL ($p < 0.01$) in the model. To examine if the pooled fish data showed a relationship between mercury contamination and $\delta^{15}N$ we constructed a model with species, $\delta^{15}N$ (size normalized for the lake trout), and the species by $N^{13}C$ interaction to predict size normalized log mercury concentration. We detected no significant interaction, so this term was removed from the model ($p = 0.88$). There was a significant relationship between size adjusted log Hg and $\delta^{15}N$ as well as species in the reduced model (Table 4). Adding depth to the model, however, resulted in an insignificant effect of $\delta^{15}N$ (Table 5).

DISCUSSION

Isotope ratio trends with depth and seasonal patterns in invertebrates

The dipterans generally showed clear relationships between the isotope ratios and the site depth. The increase in dipteran $\delta^{15}N$ with site depth during both seasons and the decline in $\delta^{13}C$ in summer appear to reflect the declining importance of benthic primary producers and the increased role of pelagic primary producers to the benthic community (Rau 1980, Hecky and Hesslein 1995, Vander Zanden and Rasmussen 1999). The dipteran winter $\delta^{13}C$ qualitatively followed a similar pattern with site depth as the summer sample, but no significant relationship was detected. Flathead Lake mixes throughout winter (except during rare periods of ice cover) which should break down

seston isotope gradients with depth in the water column, and incorporation of this seston could have weakened the relationship between the dipteran isotope ratios and site depth. The summer clam samples appeared to follow similar isotope ratios patterns as the dipterans with site depth, but the small sample size precludes drawing any firm conclusions.

The dipteran $\delta^{13}C$ and $\delta^{15}N$ patterns we observed with site depth have been documented for lentic invertebrates by others, but generally at the more discrete level of lake zones (France 1995, Vander Zanden and Rasmussen 1999). It is clear from the present work and the $\delta^{15}N$ findings of Yoshii (1999) for gammarids, however, that the isotope ratios of benthic organisms can be distributed in a continuous manner to a considerable depth. If our findings from Flathead Lake are representative, the relatively continuous isotope signal of benthic feeding invertebrates should provide a useful depth signal in large, oligotrophic lakes, but will hinder isotopic assessments that categorize lakes into distinct zones (except in deep water).

The lack of relationship between the *M. relicta* isotope ratios and depth appears to reflect the ecology of this species. In Flathead Lake adult *M. relicta* feed primarily on *Daphnia thorata* in the water column. Deep water zooplankton have a more negative $\delta^{13}C$ and a higher $\delta^{15}N$ than surface zooplankton during summer stratification (Vander Zander and Rasmussen 1999). However, *M. relicta* is thermally excluded from the epilimnion of Flathead Lake in late summer and most individuals feed at or just below the thermocline (Spencer et al. 1999). Thus *M. relicta* residing at any site depth during the day feed at a similar distance from the surface at night which could uncouple their isotope ratios from any water zooplankton isotope ratio zonation in the water column. In

the winter, mixing of the water column presumably hinders the establishment of any zooplankton isotope ratio gradients with depth which would further inhibit the formation of isotope versus site depth relationships in *M. relicta*. Further, the mobility of *M. relicta* could hinder the development of isotope versus depth patterns. The increase in *M. relicta* $\delta^{15}N$ with size appears to reflect the trophic shift in diet from diatoms, pollen, and unidentified detritus in juveniles to *Daphnia thorata* in the adults, as $\delta^{15}N$ is higher in zooplankton than phytoplankton and terrestrial vegetation (Yoshioka et al. 1988; Hecky and Hesslein 1995). Because *M. relicta* size increases with site depth (Morgan and Threlkeld 1982), *M. relicta* predators at deeper sites may nevertheless acquire a higher $\delta^{15}N$ by feeding on larger individuals.

The invertebrate carbon isotopes ratios appear to have more seasonal stability than did the nitrogen ratios. Modeling work by Johannsson et al. (2001) suggests nitrogen turnover is more rapid than carbon in *M. relicta* which corresponds to the observed patterns. $\delta^{13}C$ was relatively constant between the seasons in the dipteran samples, which may have been facilitated by the buffering effect of the benthic organic matter on isotopically variable seston inputs (Toda and Wada 1990, Gu et al. 1994, Yoshioka et al. 1994). Winter declines in *M. relicta* $\delta^{13}C$ and increases in both *M. relicta* and dipteran $\delta^{15}N$ may be influenced by seasonal shifts in the isotope ratios of diet items (Gu et al. 1994, Yoshioka et al. 1994, Grey and Jones 2001), and in the case of *M. relicta* $\delta^{15}N$ the larger size of individuals.

Habitat preference by fish

The isotope ratios in the benthic invertebrates should provide a useful depth signal for tracing habitat relationships in the higher trophic levels. However, this may not be

true for the pelagic feeding *M. relicta* unless consumption of larger individuals at deeper sites increases $\delta^{15}N$ of *M. relicta* consumers. Because lake whitefish rely heavily on dipterans and clams these fish should reflect the observed benthic invertebrate isotope ratios patterns with site depth if individuals exhibit long term foraging depth preferences. Thus the decline in lake whitefish $\delta^{13}C$ with site depth provides evidence that individual lake whitefish have long term habitat preferences. However, the lake whitefish $\delta^{15}N$ versus site depth relationship was weaker and not significant. The fish were collected in the spring after winter mixing, so stratification driven isotope gradients in the pelagic food web were probably at their weakest point which could have reduced our ability to detect foraging depth relationships fish. Further, most of the lake whitefish were captured over a relatively limited depth range which hinders detecting depth effects. Research on the same lake whitefish has shown that fish captured from deeper sites have higher mercury levels, which mimics the patterns in their food (Chapter 3). These mercury findings together with the carbon isotopes do suggest that individual lake whitefish have at least some long term depth preferences. The lake trout $\delta^{13}C$ declined with site depth and $\delta^{15}N$ increased, generally following the patterns found in the lower trophic levels. These isotope relationships together with the increasing mercury levels with depth in lake trout and their prey (Chapter 3) demonstrate that the lake trout are not mixing rapidly enough with site depth to prevent the establishment of chemical gradients in the population. The variation in the isotope ratios within fish populations has been presented as evidence of intra population diet specialization (Fry et al. 1999), which has been additionally supported by traditional stomach analysis in several studies (Gu et al. 1997, Beaudoin et al. 1999). Our results suggest that a component of the intra population

isotope variation can be comprised by feeding location preferences of individual fish in lieu of any diet differences, especially for fish relying on benthic food sources.

Trophic position versus habitat and diet

A general correspondence existed with the $\delta^{15}N$ and the trophic position among species, but reliance on different components of the lake food web apparently led to some discrepancies. For example, the dipterans from deep sites had a higher average $\delta^{15}N$ than *M. relicta* but this not supported by what is known about the trophic ecology of these animals in Flathead Lake. Adult *M. relicta* feeds mostly on primary consumers while the dipterans presumably feed on a mix of plant materials and primary consumers and thus should be of a slightly lower trophic position. Further, the summer dipteran $\delta^{15}N$ increased approximately 6‰ from the shallowest to deepest site, an increase of almost two trophic levels based on $\delta^{15}N$ (Minagawa and Wada 1984, Cabana and Rasmussen 1994) which presumably is not caused by a large trophic shift in the dipterans. These apparent discrepancies probably were caused by increases in the $\delta^{15}N$ of primary producers with depth, illustrating the need to account for differences in lower trophic levels to generate meaningful estimates of trophic position with nitrogen isotopes (Vander Zanden and Rasmussen 1999), and the need to consider spatial variation within systems.

If the $\delta^{15}N$ alone is a robust predictor of trophic position it should reflect the increasing trophic position that occurs through ontogeny in many animal populations. This was true in the *M. relicta* sample, as has been observed by others working with this species (Branstrator et al. 2000, Johannsson et al. 2001). Increases in the $\delta^{15}N$ with size often have been detected within fish species but not always (Kidd et al. 1995; Kiriluk et

al. 1995, Fry et al. 1999, Vander Zanden et al. 2000, Overman and Parrish 2001). In the lake whitefish, the lack of isotope ratio shifts through ontogeny reflects the limited diet variation within the sizes sampled. In contrast, the lake trout diet changes from predominantly *M. relicta* (secondary consumer) to lake whitefish and other fishes (secondary and tertiary consumers) (Chapter 3). Thus diet data suggest that the lake trout increase their trophic position with size, yet their $\delta^{15}N$ declines. The isotope ratios of the *M. relicta* were slightly lower than the lake whitefish, so this component of the lake trout diet shift cannot account for the observed $\delta^{15}N$ decline. However, many of the fishes consumed by the larger lake trout are littoral which have been documented to have a lower $\delta^{15}N$ than deep water species (Fry et al. 1999, Yoshii 1999). The declines in the lake trout $\delta^{15}N$ with depth could thus result from an increasing reliance of littoral production with size rather than a lower trophic position. An alternate explanation is that nitrogen isotope diet fractionation declines with size in lake trout. Other studies have reported increases, decreases, and no relationship between lake trout $\delta^{15}N$ and fish size in lakes with *M. relicta* (Kiriluk et al. 1995, Vander Zanden et al. 2000, Johnson et al. 2002). It appears that $\delta^{15}N$ alone will only generate meaningful estimates of trophic position within a species when the entire population utilizes the same nitrogen pathways (see Vander Zanden and Rasmussen 1999 Fig. 4 for a graphic representation of the problem), and $\delta^{15}N$ enrichment relative to diet does not change with size.

Isotope ratios and fish growth

We found no evidence the growth rate was related to the isotope ratios for either fish species, thus growth does not appear to be a confounding factor in our isotope analyses. $\delta^{15}N$ should be more prone to growth effects than $\delta^{13}C$ because the metabolic

fractionation of nitrogen isotopes is more substantial than for carbon. Negative relationship between $\delta^{15}N$ and growth have been reported in crabs (Fantle et al. 1999) and birds (Hobson et al. 1993), however other studies have found starvation caused no change in $\delta^{15}N$ of krill (Frazer et al. 1997) or mysids (Gorokhova and Hansson 1999).

Habitat use and trophic position versus fish mercury levels

The significant relationship between mercury contamination and foraging depth (as measured by $\delta^{13}C$) in the pooled model suggests that fish that feed at deeper sites acquire higher mercury levels. The carbon isotope patterns are in agreement with the depth results of Chapter 3 where fish captured from deeper sites were more contaminated. Together these findings illustrate how habitat use can influence fish mercury levels. In the species specific analysis the relationships between mercury and $\delta^{13}C$ were marginally significant, while in Chapter 3 using the same fish the relationships between mercury and depth of capture were substantially stronger. The weaker mercury relationships with $\delta^{13}C$ compared to depth of capture may be related to the stabilization of the carbon isotope ratios in the benthic community at deeper sites, and the depth independent $\delta^{13}C$ signal of *M. relicta*. These factors may reduce the accuracy of $\delta^{13}C$ in discriminating fish foraging depth in deep sites. Further, the timing of collection (spring) and the limited depth variation in the whitefish sample may have contributed to the lack of statistically significant mercury versus $\delta^{13}C$ relationships in the individual fish analysis.

We found that mercury contamination increased with the trophic position of fish (as measured by $\delta^{15}N$) in the pooled analysis, but not in the species specific analysis. Adding depth to the model in the pooled analysis resulted in an insignificant effect of $\delta^{15}N$. The food web of Flathead Lake is generally more contaminated with depth

(Chapter 3), thus it appears that if any relationship between $\delta^{15}N$ and contamination was likely caused by the covarying trends in mercury and $\delta^{15}N$ with site depth. Other studies have presented varying relationships between $\delta^{15}N$ and persistent contaminant levels within fish populations after accounting for size effects. Kiriluk et al. (1995) found no relationship between $\delta^{15}N$ and organic contaminant levels within a lake trout population. Bowles et al. (2001) found significant relationships between methyl mercury and $\delta^{15}N$ in five of seven fish species investigated from a tropical floodplain lake, and two of the five showed no covariation between fish length and $\delta^{15}N$. Although $\delta^{15}N$ has been use to elucidate contaminant relationships among species (Yoshinaga et al. 1992, Rolff et al. 1993, Kidd et al. 1995, Kidd et al. 1998a, Kidd 1998b, Bowles et al. 2001) and among populations (Rasmussen et al. 1990), the utility of $\delta^{15}N$ in describing contaminant variation within populations remains equivocal.

Conclusions

We demonstrate that individual fish within a population have some long term foraging depth preferences by comparing their isotope ratios to that of their diet items. We found a significant relationship between mercury contamination and average foraging depth as measured by $\delta^{13}C$ in the pooled analysis, which appears to reflect the increasing mercury levels with depth in Flathead Lake. The carbon isotope findings provide support for the hypothesis that variation in individual fish foraging (as measured by location) contributes to intra population variation in contaminant levels. We found a positive relationship between $\delta^{15}N$ and mercury in the pooled fish analysis, but interpretation of these results is obscured by correlations between mercury, depth, and $\delta^{15}N$. In general, these findings demonstrate how the ecology of individual fish can influence their

contaminant burdens within a water body, and the need to consider spatial variation in isotope ratios in food web investigations.

Acknowledgment-We thank Bonnie Ellis, Brandon Jackson, and Brooke Baldauf for assisting with invertebrate collection. The Salish-Kootenai Tribal Fisheries Program captured the fish and funded the mercury analysis. The Flathead Lake Biological Station provided boat time for invertebrate collection and funded the isotope analyses.

REFERENCES

Akielaszek, J. J., and T. A. Haines. 1981. Mercury in the muscle tissue of fish from three northern Maine lakes. Bulletin of Environmental Contamination and Toxicology 27:201-208.

Beaudoin, C. P., W. M. Tonn, E. E. Prepas, and L. I. Wassenaar. 1999. Individual specialization and trophic adaptability of northern pike (*Esox lucius*): an isotope and dietary analysis. Oecologia 120:386-396.

Bowles, K. C., S. C. Apte, W. A. Maher, M. Kawei, and R. Smith. 2001. Bioaccumulation and biomagnification of mercury in Lake Murray, Papua New Guinea. Canadian Journal of Fisheries and Aquatic Sciences 58:888-897.

Branstrator, D. K., G. Cabana, A. Mazumder, and J. B. Rasmussen. 2000. Measuring life-history omnivory in the opossum shrimp, *Mysis relicta*, with stable nitrogen isotopes. Limnology and Oceanography 45:463-467.

Bryan, J. E., and P. A. Larkin. 1972. Food specialization by individual trout. Journal of the Fisheries Research Board of Canada 29:1615-1624.

Cabana, G., and J. B. Rasmussen. 1994. Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature 372:255-257.

Campbell, L. M., D. W. Schindler, D. C. G. Muir, D. B. Donald, and K. A. Kidd. 2000. Organochlorine transfer in the food web of subalpine Bow Lake, Banff National Park. Canadian Journal of Fisheries and Aquatic Sciences 57:1258-1269.

Chess D. W., and J. A. Stanford. 1998. Comparative energetics and life cycle of the opossum shrimp (*Mysis relicta*) in native and non-native environments. Freshwater Biology 40:783-794.

Cooper, S. D., and C. R. Goldman. 1980. Opossum shrimp (*Mysis relicta*) predation on zooplankton. Canadian Journal of Fisheries and Aquatic Sciences 37:909-919.

Degens, E. T., R. R. L. Guillard, W. M. Sackett, and J. A. Hellebust. 1968. Metabolic fractionation of carbon isotopes in marine plankton-I. Temperature and respiration experiments. Deep-Sea Research 15:1-9.

DeNiro, M. J., and S. Epstein. 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197:261-263.

Ehlinger, T. J., and D. S. Wilson. 1988. Complex foraging polymorphism in bluegill sunfish. Proceedings of the National Academy of Sciences of the United States of America 85:1878-1882.

Estep, M. L. F., and S. Vigg. 1985. Stable carbon and nitrogen isotope tracers of trophic dynamics in natural populations and fisheries of the Lahontan Lake System, Nevada. Canadian Journal of Fisheries and Aquatic Sciences 42:1712-1719.

Fantle, M. S., A. I. Dittel, S. M. Schwalm, C. E. Epifanio, and M. L. Fogel. 1999. A food web analysis of the juvenile blue crab, *Callinectes sapidus*, using stable isotopes in whole animals and individual amino acids. Oecologia 120:416-426.

France, R. L. 1995. Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. Limnology and Oceanography 40:1310-1313.

France, R. L., and R. H. Peters. 1997. Ecosystem differences in the trophic enrichment of ^{13}C in aquatic food webs. Canadian Journal of Fisheries and Aquatic Sciences 54:1255-1258.

Frazer, T. K., R. M. Ross, L. B. Quetin, and J. P. Montoya. 1997. Turnover of carbon and nitrogen during growth of larval krill, *Euphausia superba* Dana: a stable isotope approach. Journal of Experimental Marine Biology and Ecology 212:259-275.

Fry, B. 1981. Natural stable carbon isotope, tag traces Texas shrimp migrations. Fishery Bulletin 79:337-345.

Fry, B., and C. Arnold. 1982. Rapid $^{13}C/^{12}C$ turnover during growth of brown shrimp (*Penaeus aztecus*). Oecologia 54:200-204.

Fry, B., P. L. Mumford, F. Tam, D. D. Fox, G. L. Warren, K. E. Havens, and A. D. Steinman. 1999. Trophic position and individual feeding histories of fish from Lake Okeechobee, Florida. Canadian Journal of Fisheries and Aquatic Sciences 56:590-600.

Gorokhova, E., and S. Hansson. 1999. An experimental study on variations in stable carbon and nitrogen isotope fractionation during growth of *Mysis mixta* and *Neomysis integer*. Canadian Journal of Fisheries and Aquatic Sciences 56:2203-2210.

Grey, J., and R. I. Jones. 2001. Seasonal changes in the importance of the source of organic matter to the diet of zooplankton in Loch Ness, as indicated by stable isotope analysis. Limnology and Oceanography 46:505-513.

Gu, B., D. M. Schell, and V. Alexander. 1994. Stable carbon and nitrogen isotopic analysis of the plankton food web in a subarctic lake. Canadian Journal of Fisheries and Aquatic Sciences 51:1338-1344.

Gu, B., C. L. Schelske, and M. V. Hoyer. 1997. Intrapopulation feeding diversity in blue tilapia: evidence from stable-isotope analyses. Ecology 78:2263-2266.

Haines, E. B., and C. L. Montague. 1979. Food sources of estuarine invertebrates analyzed using $^{13}C/^{12}C$ ratios. Ecology 60:48-56.

Hall B. D., R. A. Bodaly, R. J. P. Fudge, J. W. M. Rudd, and D. M. Rosenberg. 1997. Food as the dominant pathway of methylmercury uptake by fish. Water, Air, and Soil Pollution 100:12-24.

Hecky, R. E., and R. H. Hesslein. 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. Journal of the North American Benthological Society 14:631-653.

Hesslein, R. H., M. J. Capel, D. E. Fox, and K. A. Hallard. 1991. Stable isotopes of sulfur, carbon, and nitrogen as indicators of trophic level and fish migration in the lower Mackenzie River Basin, Canada. Canadian Journal of Fisheries and Aquatic Sciences 48:2258-2265.

Hesslein, R. H., K. A. Hallard, and P. Ramlal. 1993. Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$. Canadian Journal of Fisheries and Aquatic Sciences 50:2071-2076.

Hobson, K. A., R. T. Alisauskas, and R. G. Clark. 1993. Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implication for isotopic analysis of diet. Condor 95:388-394.

Johannsson, O. E., M. F. Leggett, L. G. Rudstam, M. R. Servos, M. A. Mohammadian, G. Gal, R. M. Dermott, and R. H. Hesslein. 2001. Diet of *Mysis relicta* in Lake Ontario as revealed by stable istope and gut content analysis. Canadian Journal of Fisheries and Aquatic Sciences 58:1975-1986.

Johnson, B. M., P. J. Martinez, and J. D. Stockwell. 2002. Tracking trophic interactions in coldwater reservoirs using naturally occurring stable isotopes. Transactions of the American Fisheries Society 131:1-13.

Juday, C., and E. A. Birge. 1927. Pontoporeia and Mysis in Wisconsin lakes. Ecology 8:445-452.

Kidd, K. A., R. H. Hesslein, R. J. P. Fudge, and K. A. Hallard. 1995. The influence of trophic level as measured by $\delta^{15}N$ on mercury concentrations in freshwater organisms. Water, Air, and Soil Pollution 80:1011-1015.

Kidd, K. A., R. H. Hesslein, B. J. Ross, K. Koczanski, G. R. Stephens, and D. C. G Muir. 1998a. Bioaccumulation of organochlorines through a remote freshwater food web in the Canadian Arctic. Environmental Pollution 102:91-103.

Kidd, K.A., D. W. Schindler, R. H. Hesslein, and D. C. G. Muir. 1998b. Effects of trophic position and lipid on organochlorine concentrations in fishes from subarctic lakes in Yukon Territory. Canadian Journal of Fisheries and Aquatic Sciences 55:869-881.

Kiriluk, R. M., M. R. Servos, D. M. Whittle, G. Cabana, and J. B. Rasmussen. 1995. Using ratios of stable nitrogen and carbon isotopes to characterize the biomagnification of DDE, mirex, and PCB in a Lake Ontario pelagic food web. Canadian Journal of Fisheries and Aquatic Sciences 52:2660-2674.

Langeland, A., J. I. Koksvik, and J. Nydal. 1991. Impact of the introduction of *Mysis relicta* on the zooplankton and fish populations in a Norwegian lake. American Fisheries Society Symposium 9:98-114.

Lasenby, D. C., T. G. Northcote, and M. Fürst. 1986. Theory, practice, and effects of *Mysis relicta* introductions to North American and Scandinavian lakes. Canadian Journal of Fisheries and Aquatic Sciences 43:1277-1284.

Madenjian, C. P., S. R. Carpenter, G. W. Eck, and M. A. Miller. 1993. Accumulation of PCBs by lake trout (*Salvelinus namaycush*): an individual-based model approach. Canadian Journal of Fisheries and Aquatic Sciences 50:97-109.

Madenjian, C. P., S. R. Carpenter, and P. S. Rand. 1994. Why are the PCB concentrations of salmonine individuals from the same lake so highly variable? Canadian Journal of Fisheries and Aquatic Sciences 51:800-807.

McConnaughey, T., and C. P. McRoy. 1979. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. Marine Biology 53:257-262.

Minagawa, M., and E. Wada. 1984. Stepwise enrichment of 15N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochimica et Cosmochimica Acta 48:1135-1140.

Morgan, M. D., and S. T. Threlkeld. 1982. Size dependent horizontal migration of *Mysis relicta*. Hydrobiologia 93:63-68.

Oana, S., and E. S. Deevey. 1960. Carbon 13 in lake waters, and its possible bearing on paleolimnology. American Journal of Science 258-A:253-272.

Overman, N. C., and D. L. Parrish. 2001. Stable isotope composition of walleye: ^{15}N accumulation with age and area-specific differences in $\delta^{13}C$. Canadian Journal of Fisheries and Aquatic Sciences 58:1253-1260.

Potter, D. S., and J. A. Stanford. 1974. Influences on the plankton communities of oligotrophic Flathead Lake. Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie 19:1790-1797.

Rasmussen, J. B., D. J. Rowan, D. R. S. Lean, and J. H. Carey. 1990. Food chain structure in Ontario lakes determines PCB levels in lake trout (*Salvelinus namaycush*) and other pelagic fish. Canadian Journal of Fisheries and Aquatic Sciences 47: 2030-2038.

Rau, G. 1978. Carbon-13 depletion in a subalpine lake: carbon flow implications. Science 201:901-902.

Rau, G. 1980. Carbon-13/carbon-12 variation in subalpine lake aquatic insects: food source implications. Canadian Journal of Fisheries and Aquatic Sciences 37:742-746.

Rolff, C., D. Broman, C. Näf, and Y. Zebühr. 1993. Potential biomagnification of PCDD/Fs - new possibilities for quantitative assessment using stable isotope trophic position. Chemosphere 27:461-468.

Schindler, D. E., J. R. Hodgson, and J. F. Kitchell. 1997. Density-dependent changes in individual foraging specialization of largemouth bass. Oecologia 110:592-600.

Spencer, C. N, B. R. McClelland, and J. A. Stanford. 1991. Shrimp stocking, salmon collapse, and eagle displacement. BioScience 41:14-21.

Spencer, C. N., D. S. Potter, R. T. Bukantis, and J. A. Stanford. 1999. Impact of predation by *Mysis relicta* on zooplankton in Flathead Lake, Montana, USA. Journal of Plankton Research 21:51-64.

Stanford, J. A., and B. K. Ellis. In press. Natural and cultural influences on ecosystems processes in the Flathead River Basin (Montana, British Columbia). Chapter 14 *in* J. S. Baron, editor. Rocky Mountain futures: an ecological perspective. Island Press, Covelo, California, USA.

Stanford, J. A., and F. R. Hauer. 1992. Mitigating the impacts of stream and lake regulation in the Flathead River catchment, Montana, USA: an ecosystem perspective. Aquatic Conservation: Marine and Freshwater Ecosystems 2:35-63.

Toda, H., and E. Wada. 1990. Use of $^{15}N/^{14}N$ rations to evaluate the food source of the mysid, *Neomysis intermedia* Czerniawsky, in a eutrophic lake in Japan. Hydrobiologia 194:85-90.

Vander Zanden, M. J., and J. B. Rasmussen. 1996. A trophic position model of pelagic food webs: impact on contaminant bioaccumulation in lake trout. Ecological Monographs 66:451-477.

Vander Zanden, M. J., and J. B. Rasmussen. 1999. Primary consumers $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. Ecology 80:1395-1404.

Vander Zanden, M. J., B. J. Shuter, N. P. Nigel, and J. B. Rasmussen. 2000. Within- and among-population variation in the trophic position of a pelagic predator, lake trout

(*Salvelinus namaycush*). Canadian Journal of Fisheries and Aquatic Sciences 57:725-731.

Yoshii, K. 1999. Stable isotope analyses of benthic organisms in Lake Baikal. Hydrobiologia 411:145-159.

Yoshioka, T., E. Wada, and H. Hayashi. 1994. A stable isotope study on seasonal food web dynamics in a eutrophic lake. Ecology 75: 835-846.

Yoshioka, T., E. Wada, and Y. Saijo. 1988. Analysis of lacustrine food web with natural carbon and nitrogen isotope ratios. Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie 23:573-578.

Yoshinaga, J., T. Suzuki, T. Hongo. M. Minagawa, R. Ohtsuka, T. Kawabe, T. Inaoka, and T. Akimichi. 1992. Mercury concentration correlates with the nitrogen stable isotope ratio in the animal food of Papuans. Ecotoxicology and Environmental Safety 24:37-45.

Table 1. Lake trout $\delta^{15}N$ as predicted by multiple regression of depth and TL.

Model R^2	Predictor	B	p
0.45	constant	11.8	
	TL	-0.00121	0.01
	depth	0.00735	0.01

Table 2. Lake trout $\delta^{15}N$ as predicted by multiple regression of TL and $\delta^{13}C$.

Model R^2	Predictor	B	p
0.68	constant	5.74	
	TL	-0.00174	<0.01
	$\delta^{13}C$	-0.228	<0.01

Table 3. Size adjusted log mercury concentration versus species and $\delta^{13}C$ for pooled lake trout and lake whitefish sample.

Model R^2	Predictor	B	p
0.82	constant	1.72	
	species	-0.536	0.04
	$\delta^{13}C$	-0.0310	<0.01

Table 4. Size adjusted log mercury concentration versus species and $\delta^{15}N$ (size adjusted for lake trout) for pooled lake trout and lake whitefish sample.

Model R^2	Predictor	B	p
0.83	constant	1.84	
	species	-0.299	<0.01
	$\delta^{15}N$	0.0701	0.02

Table 5. Size adjusted log mercury concentration versus species, $\delta^{15}N$ (size adjusted for lake trout), and depth for pooled lake trout and lake whitefish sample.

Model R^2	Predictor	B	p
0.85	constant	2.05	
	species	-0.353	<0.01
	$\delta^{15}N$	0.0459	0.11
	depth		0.01

Fig. 1. Dipteran and clam $\delta^{15}N$ versus depth of capture during summer and winter.

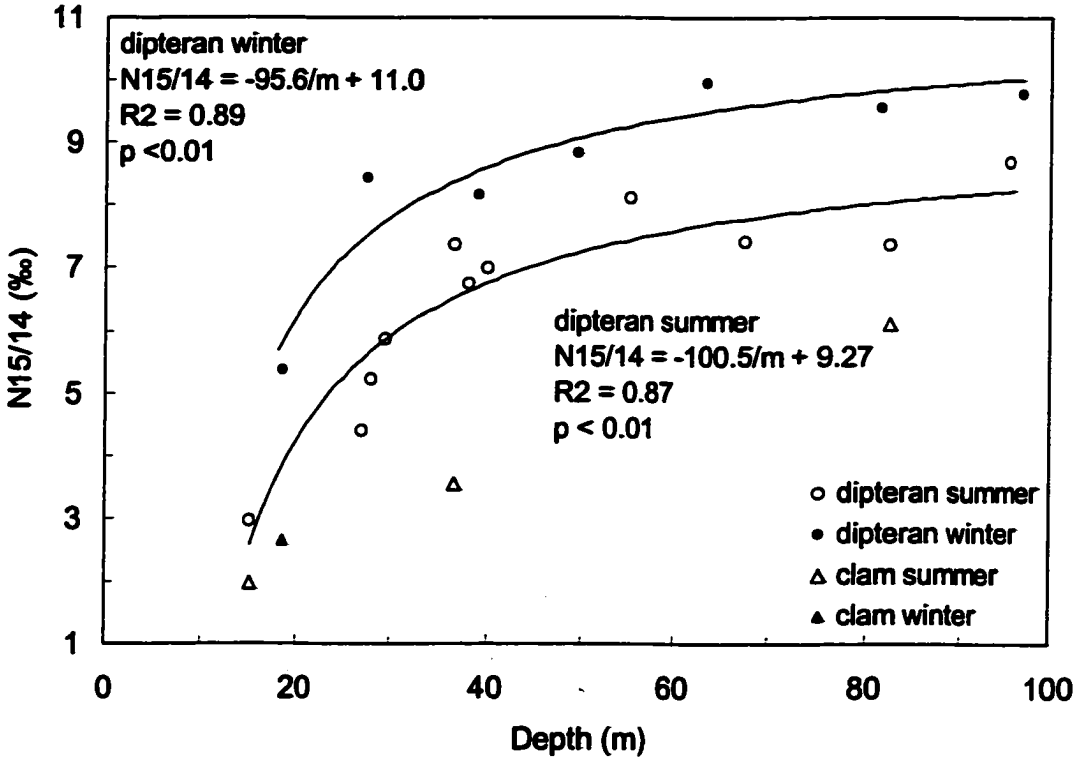


Fig. 2. Dipteran and clam $\delta^{13}C$ versus depth of capture during summer and winter.

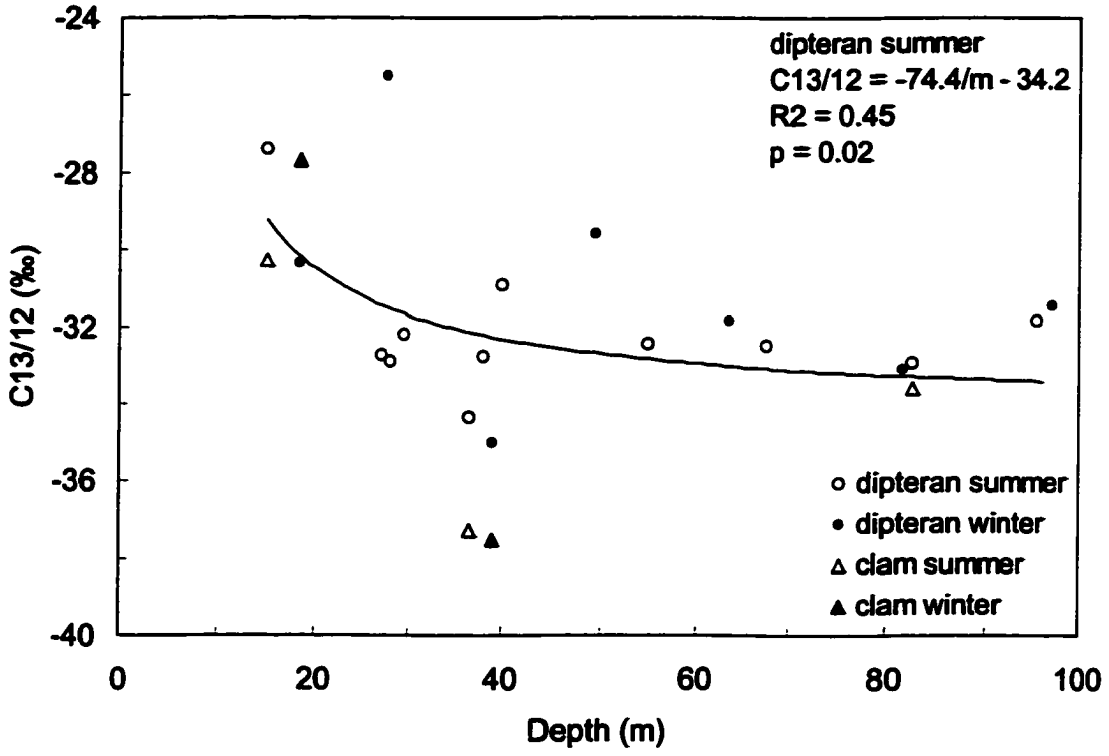


Fig. 3. *Mysis relicta* $\delta^{15}N$ versus depth of capture during summer and winter.

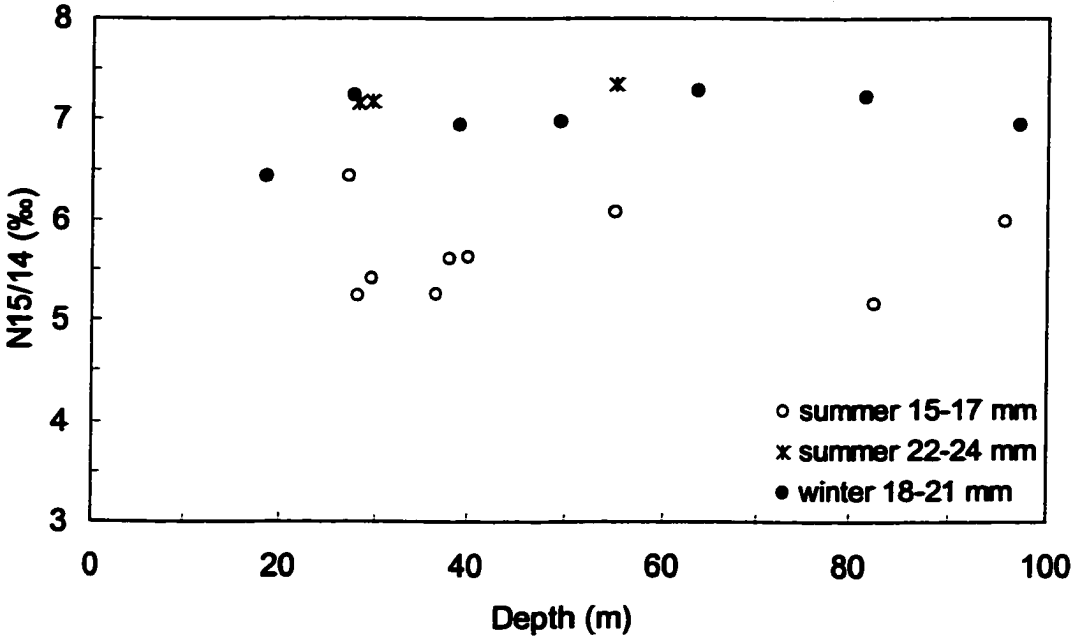


Fig. 4. *Mysis relicta* $\delta^{13}C$ versus depth of capture during summer and winter.

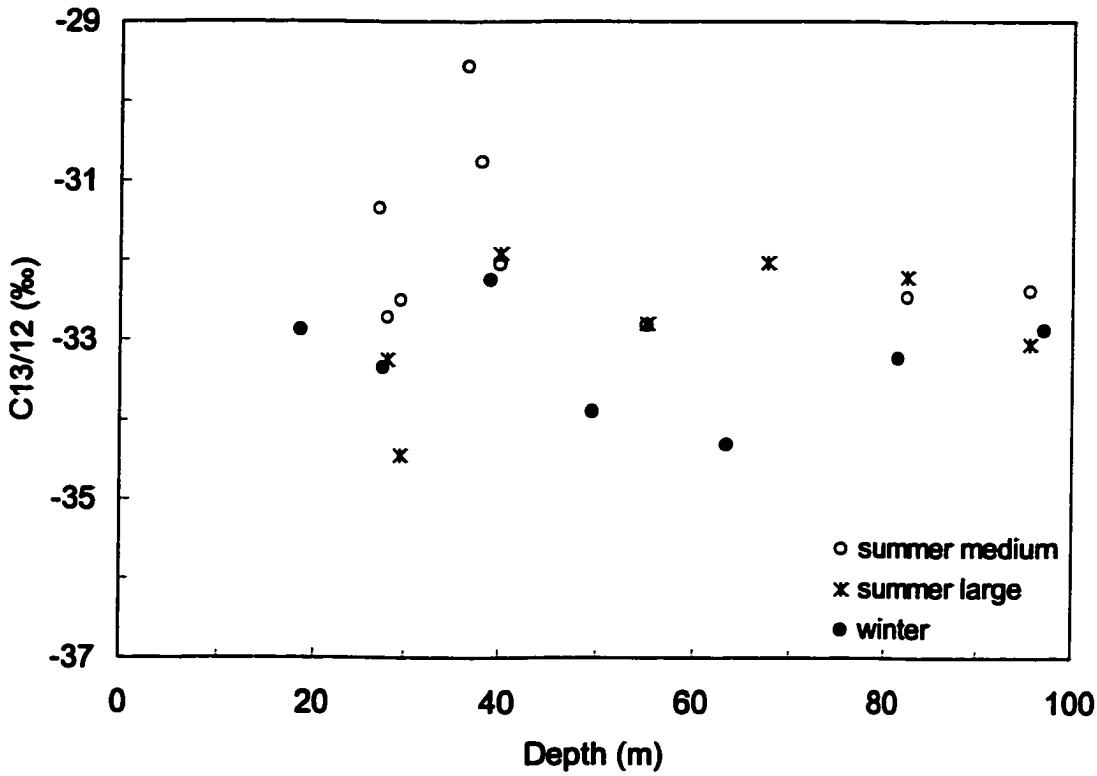


Fig. 5. $\delta^{15}N$ and $\delta^{13}C$ versus *M. relicta* length from a 55 m deep site.

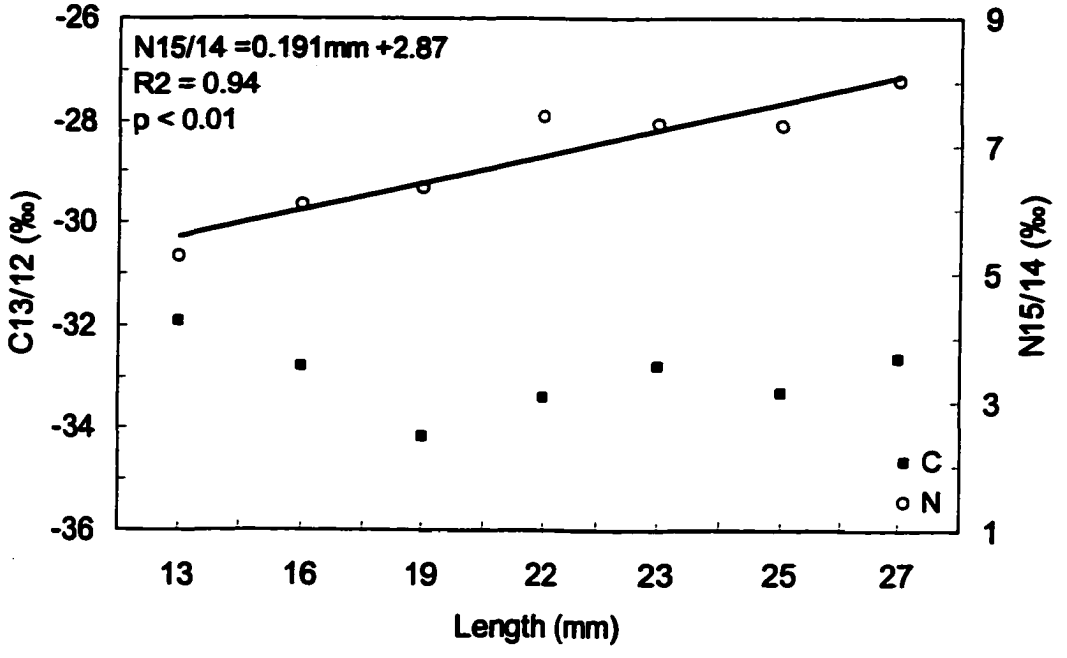


Fig. 6. Lake trout $\delta^{15}N$ versus depth of capture.

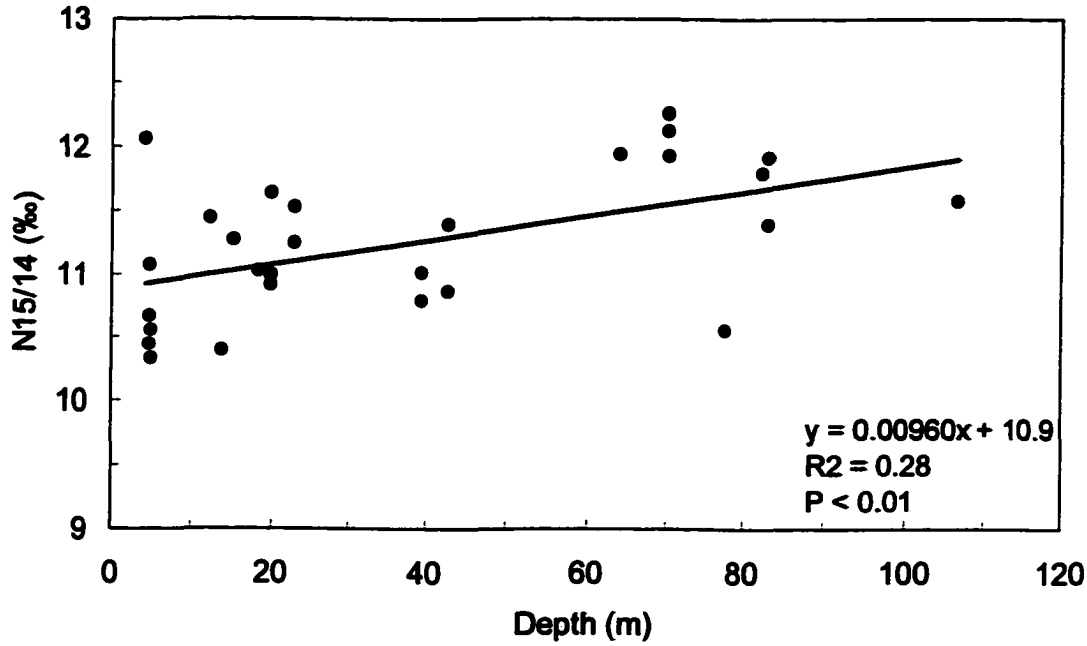


Fig. 7. Lake whitefish $\delta^{15}N$ versus site depth.

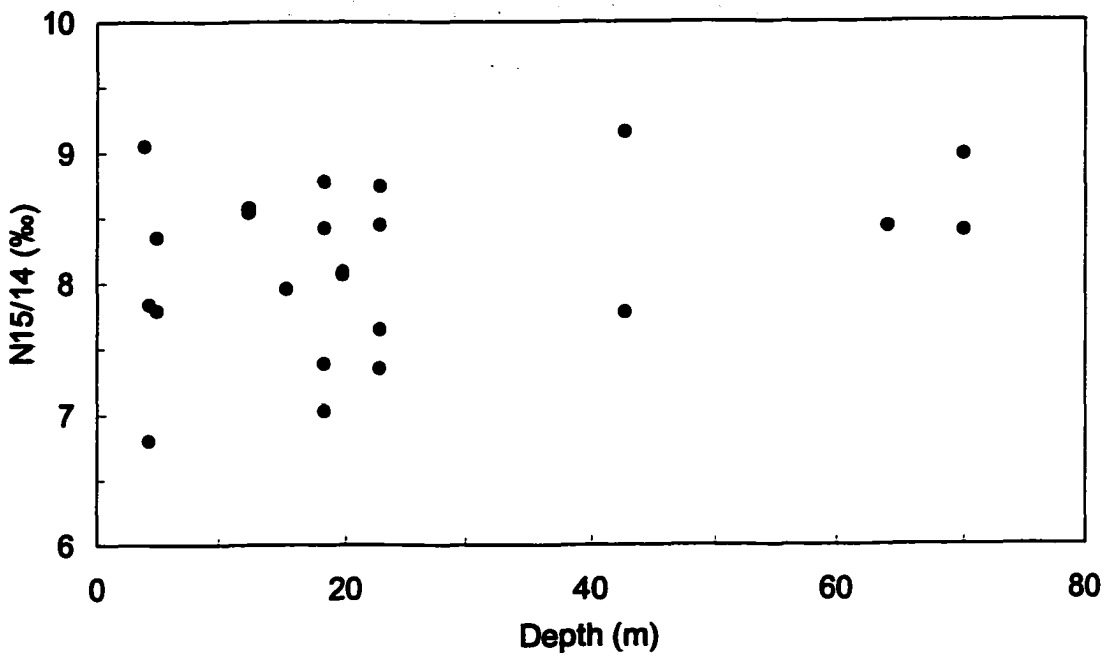


Fig. 8. Lake trout $\delta^{13}C$ versus site depth.

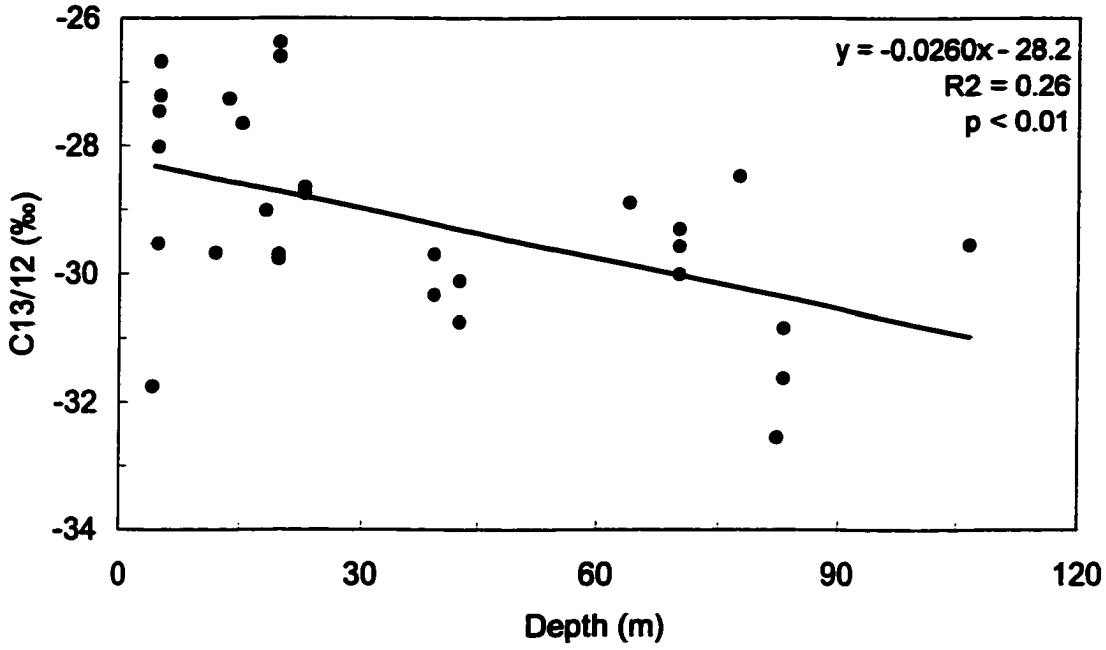
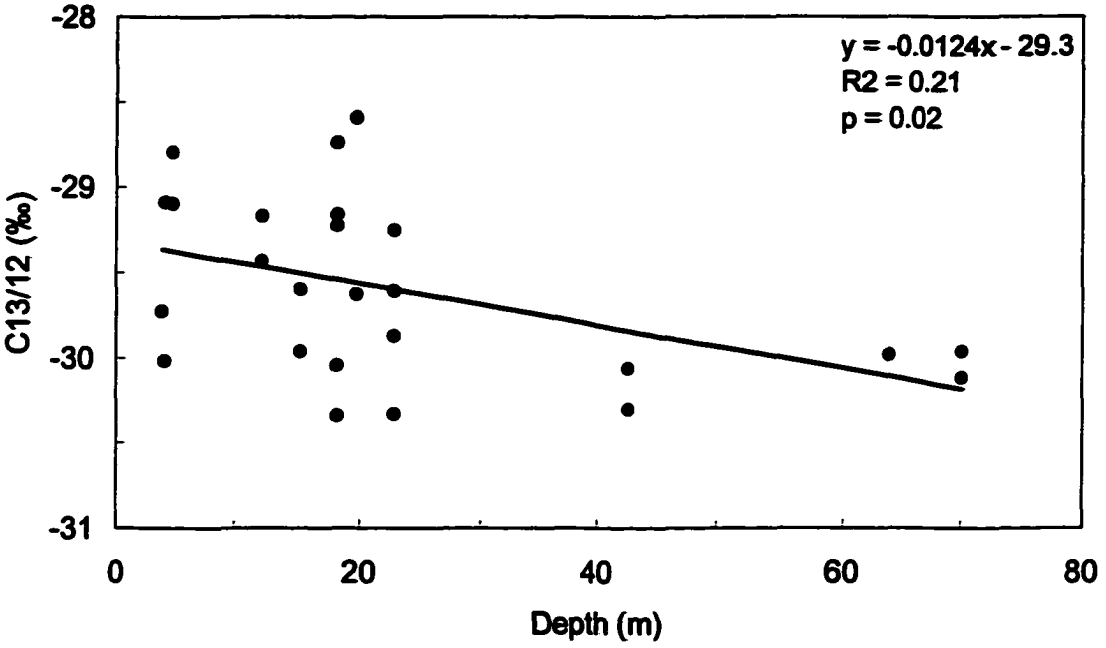


Fig. 9. Lake whitefish $\delta^{13}C$ versus depth of capture.



CHAPTER 5

EFFECTS OF MYSIS PREDATION ON ZOOPLANKTON VERTICAL DISTRIBUTION

Craig P. Stafford and Jack A. Stanford

ABSTRACT

We investigated the vertical distribution of zooplankton in Montana lakes with and without the introduced zooplankton predator *Mysis relicta* during summer stratification. Lakes with Mysis had a greater proportion of cladocerans in the surface waters, but no differences in the vertical zonation of rotifers, nauplii, or copepods (excluding nauplii) were detected. The overall composition of zooplankton was similar in lakes with and without Mysis which may have been influenced by the zooplankton thermal refugia from Mysis in the epilimnion. We conclude that Mysis predation of zooplankton is only one of several factors which interact to structure the vertical distribution of zooplankton communities.

INTRODUCTION

In lentic ecosystems large food web changes have occurred after the introduction of the shrimp *Mysis relicta* Lovén (Northcote, 1991; Spencer et al. 1991). Mysis is cold water species native to northern Eurasia as well as north central and northeast North America (Dadswell, 1974). The range of Mysis was expanded substantially by fisheries

managers who introduced Mysis to numerous lakes and reservoirs in Scandinavia and western North America (Fürst, 1981; Lasenby et al., 1986).

Mysis introductions largely were inspired by the increased growth and sustained abundance of kokanee (*Oncorhynchus nerka*) after plantings of Mysis in Kootenay Lake, British Columbia (Sparrow et al., 1964; Northcote, 1972). However these results generally have not been duplicated elsewhere (Morgan et al., 1978; Lasenby et al. 1986; Beattie and Clancey, 1991; Bowles et al., 1991), and appear to be caused by the peculiar morphometry and nutrient enrichment of Kootenay Lake (Northcote, 1972; Martin & Northcote, 1991).

Mysis consumes a variety of food sources (Grossnickle, 1982; Johannsson et al., 2001), but adults generally prefer cladocerans when available (Cooper & Goldman, 1980; Bowers & Vanderploeg, 1982; Chess and Stanford, 1998; Spencer et al., 1999). Mysis feeds at night in the water column and returns to the bottom during the day (Juday & Birge, 1927), limiting predation by pelagic fishes (Hammar, 1988; Bowles et al., 1991) and increasing metabolic efficiency (Chess and Stanford, 1999). Cladoceran densities generally have declined after Mysis introductions (Richards et al., 1975; Spencer et al., 1999). However, the impact of Mysis on cladocerans is mediated by lake morphometry. Mysis select water temperatures of approximately 15-16 °C or colder (Levy, 1991; Spencer et al., 1999), thus the epilimnion of lakes can serve as a thermal refuge for zooplankton prey (Nero & Sprules, 1986; Lehman et al., 1990).

The vertical discontinuity in Mysis predation during summer stratification may secondarily influence the vertical distribution of other zooplankton species through competitive interactions. According to the size selective hypothesis of Brooks and

Dodson (1965) the abundance of large bodied zooplankton reflects the balance between predation and metabolic efficiency. Large bodied species are more susceptible to predators but are also metabolically more efficient, therefore large bodied species should proliferate when predation risk is low. Accordingly Mysis predation of cladocerans below the epilimnion could release populations of smaller zooplankton species, however this may not occur in the epilimnion when Mysis is thermally excluded. Thus stocking of Mysis to lakes provides an opportunity to examine the size selective hypothesis during summer stratification at the whole lake scale.

In the current investigation we documented the vertical distribution of zooplankton during summer stratification using a replicated whole lake design that incorporated seven relatively similar oligotrophic lakes. Our primary hypothesis is that during summer stratification Mysis predation of deep water zooplankton creates a fundamental dichotomy in the vertical distribution of zooplankton. Specifically, we expected that cladocerans would be relatively more abundant in the epilimnion in response to Mysis predation which may cause competitive release of small bodied and highly mobile zooplankton in deep water.

METHODS

The seven study lakes lie within the upper Columbia River drainage in northwest Montana. In general these lakes are relatively large, deep, and oligotrophic. Between 1968-1975 Mysis were introduced from Waterton Lake on the east side of continental divide to 12 lakes in northwest Montana on the west side of the continental divide (Spencer et al., 1991). Mysis established in several lakes, including three of the study

lakes (Ashley, Swan, and Whitefish) and a fourth study lake was colonized via downstream drift (Flathead Lake). Three study lakes remain without Mysis (Lindbergh, McDonald, and Tally). Lindberg Lake and Lake McDonald were never stocked with Mysis. Tally Lake apparently was stocked unsuccessfully, as a noctural survey with a 0.79 m^2 500 μm mesh Wisconsin style net on August 2, 1999 failed to capture any specimens.

Each lake was sampled four times at its deepest point during summer stratification from July 29 to August 4, 1999, July 17 to 23, 1999, July 27 to 31, 2000, and August 20 to 25, 2000. Lakes were characterized for Secchi depth and total phosphorous on every visit, and profiles of temperature, conductivity, and pH were taken with a Hydrolab Surveyor III in 2000. Duplicate zooplankton hauls were made from the depth corresponding to 15 °C to the surface, and a second set of hauls were taken from 10 m below the depth corresponding to 15 °C to 15 °C. Hauls were made with a 0.29 m^2 64 μm mesh Wisconsin style net and samples were preserved in 70% ethanol. Zooplankton were enumerated under a compound microscope using a 1 ml counting cell, except cladocerans from Swan Lake were so rare in 1999 it was necessary to count them with a dissecting scope using 5 ml aliquots. We identified cladocerans to genus, copepods to order (but pooled nauplii), and pooled all rotifers. To facilitate comparisons amongst the lakes, these categories were further pooled into cladocerans, copepods (excluding nauplii), nauplii, or rotifers. To characterize the vertical zonation in the zooplankton community we calculated the percentage of surface zooplankton (% surface) as: 100*(density surface haul)/(density surface haul + density deep haul). The overall zooplankton composition was characterized for each lake by summing zooplankton

densities of the surface and deep hauls for each sampling date and expressing each taxonomic category as a percentage of the total summed zooplankton for that date. To examine if the zooplankton % surface and overall composition varied with Mysis presence or absence, we used repeated measures ANOVA.

RESULTS

The lakes varied in their physical and chemical characteristics, but no significant differences were detected between lakes with and without Mysis using t-tests ($p \geq 0.14$ for all comparisons) (Table 1). Cladocerans were comprised of Bosmina, Ceriodaphnia, Daphnia, Holopedium, and Polyphemus, but the composition varied widely among lakes. Calanoids were present in all lakes to varying degrees and generally outnumbered by cyclopoids. Nauplii were less abundant than later copepod stages. Rotifers were the most abundant category in all lakes (Table 2). Repeated measures ANOVA indicated % surface cladocerans was significantly higher in the presence of Mysis ($p = 0.05$), while no significant differences were observed for rotifers ($p = 0.25$), copepods ($p = 0.92$), or nauplii ($p = 0.06$) (Figure 1). No significant differences in the overall zooplankton composition were detected for cladocerans ($p = 0.56$), rotifers ($p = 0.12$), copepods ($p = 0.10$), or nauplii ($p = 0.35$) (Figure 2).

DISCUSSION

We believe the use of a replicated whole lake design improves our ability to attribute any differences in the zooplankton communities to the effect of Mysis. Although Mysis were not randomly applied to the lakes in the strictest sense, lake groups

with and without Mysis did not vary detectably in the measured chemical and physical attributes supporting our contention that any differences in the zooplankton community most likely were Mysis induced.

The greater proportion of surface cladocerans in Mysis lakes strongly suggests that Mysis predation interacts with lake thermal conditions to structure the vertical distribution of cladocerans. These findings are in general agreement with several pre versus post Mysis (Richards et al., 1975; Spencer et al., 1991) and lake comparative studies (Langeland et al., 1991A; Almond et al., 1996) that suggest Mysis reduces cladocerans densities or biomass in the water column. Specifically our findings support Nero & Sprules (1986) who found lower densities of deep water cladocerans in lakes with native Mysis as well as Spencer et al. (1999) who found reduced densities of cladocerans in the hypolimnion of Flathead Lake after Mysis establishment. Our results are also in accordance with several studies that have reported a thermal refuge for Mysis prey in the epilimnion (Nero & Sprules, 1986; Lehman et al., 1990). We were unable to detect differences in the overall composition of the zooplankton groups with Mysis presence which may have been related to the timing of the zooplankton collections. The samples were collected during late summer when the thermal refuge was at its maximum which may have limited our ability to detect Mysis induced differences in the overall zooplankton composition.

Contrary to our expectations, we were unable to demonstrate a greater proportion of deep water copepods, rotifers, or nauplii associated with the Mysis predation of deep water cladocerans. Relative to cladocerans, copepods generally are smaller and much faster moving. Nevertheless, Mysis predation on copepods has been documented

(Cooper & Goldman, 1980; Johannsson et al., 2001), particularly when cladocerans are at low densities (Chess & Stanford, 1998). Thus is possible that any competitive release of copepods via the loss of cladocerans was offset by direct mortality caused by Mysis predation. Declines in copepods have been reported after Mysis introductions in some systems (Koksvik et al., 1991; Spencer et al., 1999) but no obvious change has been detected in others (Langeland et al., 1991B; Koksvik et al., 1991). We were particularly surprised that the proportion of deep water rotifers was not increased via the loss of deep water cladocerans. Several studies have noted an increase in rotifer abundance in response to the loss of larger bodied zooplankton (Qin and Culver, 1996; Sondergaard et al., 1997), although Koksvik et al. (1991) also found no change in the rotifer component of the zooplankton community (as measured by biomass) after Mysis establishment. Consumption of rotifers by Mysis has been documented (Martinez & Bergersen, 1991; Johannsson et al., 2001), however we feel it is unlikely that Mysis predation on these abundant and rapidly reproducing organisms is sufficient to substantially affect the overall abundance of the rotifer community. Our inability to document a release of copepods and rotifers in Mysis lakes may in part reflect the variation in the zooplankton community between the study lakes, especially given our relatively small sample size of lakes. These differences reflect the many factors that shape zooplankton communities such as water chemistry, lake physical conditions, food quality and quantity, zooplankton species pool, and the abundance and composition of the fish community. This variation necessitated compressing many species into a few broad categories which may have hindered our ability to detect differences. Further, vertical movement of zooplankton may have obscured our ability to detect any competitive release of non cladoceran taxa.

In general these results illustrate how lake physical conditions interact with the ecology of lentic biota to structure lake food webs. Specifically, we illustrate how Mysis predation of deep water cladocerans vertically structures lake food webs during summer stratification. We found no evidence that the loss of deep water cladocerans released populations smaller or rotifers or copepods. We conclude that Mysis predation of zooplankton is one of myriad of factors which shape zooplankton communities and their vertical distribution in our study lakes.

Acknowledgement-We thank Levia Jones, Angelika Buscha, Andi Shockley, Vicki Ludden, Samantha Chilcote, David Phillips, Jake Chaffin, and Mary Harner for assistance with field work.

REFERENCES

Almond M.J.R., Bentzen E. & Taylor W.D. (1996) Size structure and species composition of plankton communities in deep Ontario lakes with and without *Mysis relicta* and planktivorous fish. *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 315-325.

Beattie W.D. & Clancey P.T. (1991) Effects of *Mysis relicta* on the zooplankton community and kokanee population of Flathead Lake, Montana. *American Fisheries Society Symposium*, **9**, 39-48.

Bowers, J.A. & Vanderploeg, H.A. (1982) *In situ* predatory behavior of *Mysis relicta* in Lake Michigan. *Hydrobiologia*, **93**, 121-131.

Bowles E.C., Rieman B.E., Mauser G.R. & Bennett D.H. (1991) Effects of introductions of *Mysis relicta* on fisheries in northern Idaho. *American Fisheries Society Symposium*, **9**, 65-74.

Brooks J.L. & Dodson S.I. (1965) Predation, body size, and composition of plankton. *Science*, **150**, 28-35.

Chess D.W. & Stanford J.A. (1998) Comparative energetics and life cycle of the opossum shrimp (*Mysis relicta*) in native and non-native environments. *Freshwater Biology*, **40**, 783-794.

Chess D.W. & Stanford J.A. (1999) Experimental effects of temperature and prey assemblage on growth and lipid accumulation by *Mysis relicta* loven. *Hydrobiologia*, **412**, 155-164.

Cooper S.D. & Goldman C.R. (1980) Opossum shrimp (*Mysis relicta*) predation on zooplankton. *Canadian Journal of Fisheries and Aquatic Sciences*, **37**, 909-919.

Dadswell M.J. (1974) Distribution, ecology, and postglacial dispersal of certain crustaceans and fishes in eastern North America. National Museum of Natural Science, Publications in Zoology No. 11, Ottawa, Ontario.

Hammar J. (1988) Planktivorous whitefish and introduced *Mysis relicta*: ultimate competitors in the pelagic community. *Finnish Fisheries Research*, **9**, 497-521.

Fürst M. (1981) Results of introductions of new fish food organisms into Swedish lakes. *Institute of Freshwater Research Drottningholm*, **59**, 33-47.

Grossnickle N.E. (1982) Feeding habitats of *Mysis relicta* – an overview. *Hydrobiolgia*, **93**, 101-107.

Johannsson O.E., Leggett M.F., Rudstam L.G., Servos M.R., Mohammadian M.A., Gal G., Dermott R.M. & Hesslein R.H. (2001) Diet of *Mysis relicta* in Lake Ontario as revealed by stable isotope and gut content analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, **58**, 1975-1986.

Juday C. & Birge E.A. (1927) Pontoporeia and Mysis in Wisconsin Lakes. *Ecology*, **8**, 445-452.

Langeland A., Carl L.M., Hicks F.J. & Monroe B. (1991A) Impact of predation by *Mysis relicta* and fish on zooplankton in four oligotrophic, north temperate lakes. *American Fisheries Society Symposium*, **9**, 88-97.

Langeland A., Koksvik J.I., & Nydal J. (1991B) Impact of the introduction of *Mysis relicta* on the zooplankton and fish populations in a Norwegian lake. *American Fisheries Society Symposium*, **9**, 98-114.

Lasenby D.C., Northcote T.G. & Fürst M. (1986) Theory, practice, and effects of *Mysis relicta* introductions to North American and Scandinavian lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, **43**, 1277-1284.

Lehman J.T., Bowers J.A., Gensemer R.W., Warren G.J. & Branstrator D.K. (1990) *Mysis relicta* in Lake Michigan: abundances and relationships with their potential prey, Daphnia. *Canadian Journal of Fisheries and Aquatic Sciences*, **47**, 977-983.

Levy D.A. (1991) Acoustic analysis of diel vertical migration behavior of *Mysis relicta* and kokanee (*Oncorhynchus nerka*) within Okanagan Lake, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences*, **48**, 67-72.

Martin A.D. & Northcote T.G. (1991) Kootenay Lake: an inappropriate model for *Mysis relicta* introduction in north temperate lakes. *American Fisheries Society Symposium*, **9**, 23-29.

Martinez P.J. & Bergersen E.P. (1991) Interactions of zooplankton, *Mysis relicta*, and kokanees in Lake Granby, Colorado. *American Fisheries Society Symposium*, **9**, 49-64.

Morgan M.D., Threlkeld S.T. & Goldman C.R. (1978) Impact of the introduction of kokanee and opossum shrimp on a subalpine lake. *Journal of the Fisheries Research Board of Canada*, **35**, 1572-1579.

Nero, R.W. & Sprules W.G. (1986) Zooplankton species abundance and biomass in relation to occurrence of *Mysis relicta* (Malacostraca: Mysidacea). *Canadian Journal of Fisheries and Aquatic Sciences*, **43**, 420-434.

Northcote T.G. (1972) Some effects of mysid introduction and nutrient enrichment on an oligotrophic lake and its salmonids. *Internationale Vereinigun für theoretische und angewandte Limnologie Verhandlungen*, **18**, 1096-1106.

Northcote T.G. (1991) Success, problems, and control of introduced mysid populations in lakes and reservoirs. *American Fisheries Society Symposium*, **9**, 5-16.

Qin J. & Culver D.A. (1996) Effect of larval fish and nutrient enrichment on plankton dynamics in experimental ponds. *Hydrobiologia*, **321**, 109-118.

Richards R.C., Goldman C.R., Frantz T.C. & Wickwire R. (1975) Where have all the Daphnia gone? The decline of a major cladoceran in Lake Tahoe, California-Nevada. *Internationale Vereinigun für theoretische und angewandte Limnologie Verhandlungen*, **19**, 835-842.

Sondergaard M., Jeppesen E. & Berg S. (1997) Pike (*Esox lucius* L.) stocking as a biomanipulation tool 2. Effects on lower trophic levels in Lake Lyng, Denmark. *Hydrobiologia* **342/343**, 319-325.

Sparrow R.A.H., Larkin P.A. & Rutherglen R.A. (1964) Successful introduction of *Mysis relicta* Lovén into Kootenay Lake, British Columbia. *Journal of the Fisheries Research Board of Canada*, **21**, 1325-1327.

Spencer C.N., McClelland B.R. & Stanford J.A. (1991) Shrimp stocking, salmon collapse, and eagle displacement. *BioScience*, **41**, 14-21.

Spencer C.N., Potter D.S., Bukantis R.T. & Stanford J.A. (1999) Impact of predation by *Mysis relicta* on zooplankton in Flathead Lake, Montana, USA. *Journal of Plankton Research*, **21**, 51-64.

Table 1. Average Secchi depth, total phosphorous, temperature at one meter, conductivity at one meter, pH at one meter, and maximum lake depth for lakes without and with Mysis used in this study. No significant differences were observed between groups for any of the characteristics using t-tests ($p \geq 0.14$ for all comparisons).

Group	Lake	Secchi (m)	T.P. (µg/g)	Temp. (°C)	Cond. (µS/cm)	pH	Depth (m)
No Mysis	Lindberg	7.3	4.0	19.9	36	7.9	38
	McDonald	13.8	2.7	19.2	99	8.2	142
	Tally	5.9	9.4	22.5	189	8.5	150
	mean	**9.0**	**5.4**	**20.5**	**108**	**8.2**	**110**
Mysis	Ashley	8.6	9.0	20.0	253	8.5	66
	Flathead	11.0	4.6	21.0	166	8.6	116
	Swan	8.2	4.6	18.7	162	8.3	40
	Whitefish	9.5	5.4	19.7	169	8.4	68
	mean	**9.3**	**5.9**	**19.9**	**187**	**8.5**	**73**

Table 2. Overall zooplankton percent composition for combined surface and deep hauls calculated within lakes for each sampling event (n = 4) and subsequently averaged. Bos = Bosmina, Daph = Daphnia, Cerio = Ceriodaphnia, Hol = Holopedium, Poly = Polyphemus, Cal = Calanoida, Cyc = Cyclopoida, Naup = nauplii, and Rot. = Rotifera.

	Bos.	Daph.	Cerio.	Holo.	Poly.	Cal.	Cyc.	Naup.	Rot.
Ashley	3.4	2.0	6.5	0.0	0.0	0.1	20.3	14.5	53.2
Flathead	0.2	6.5	0.0	0.0	0.0	9.7	25.6	15.8	42.2
Swan	0.7	2.3	0.0	0.0	0.0	8.0	14.2	24.3	50.4
Whitefish	0.6	2.9	0.0	0.0	0.0	0.4	27.7	7.4	61.0
Lindberg	0.5	5.7	0.1	4.2	0.0	0.2	15.1	3.1	71.1
McDonald	0.5	6.1	0.0	0.0	0.0	8.5	6.7	7.9	70.4
Tally	0.2	6.5	0.0	0.0	0.1	8.6	14.0	18.3	52.3

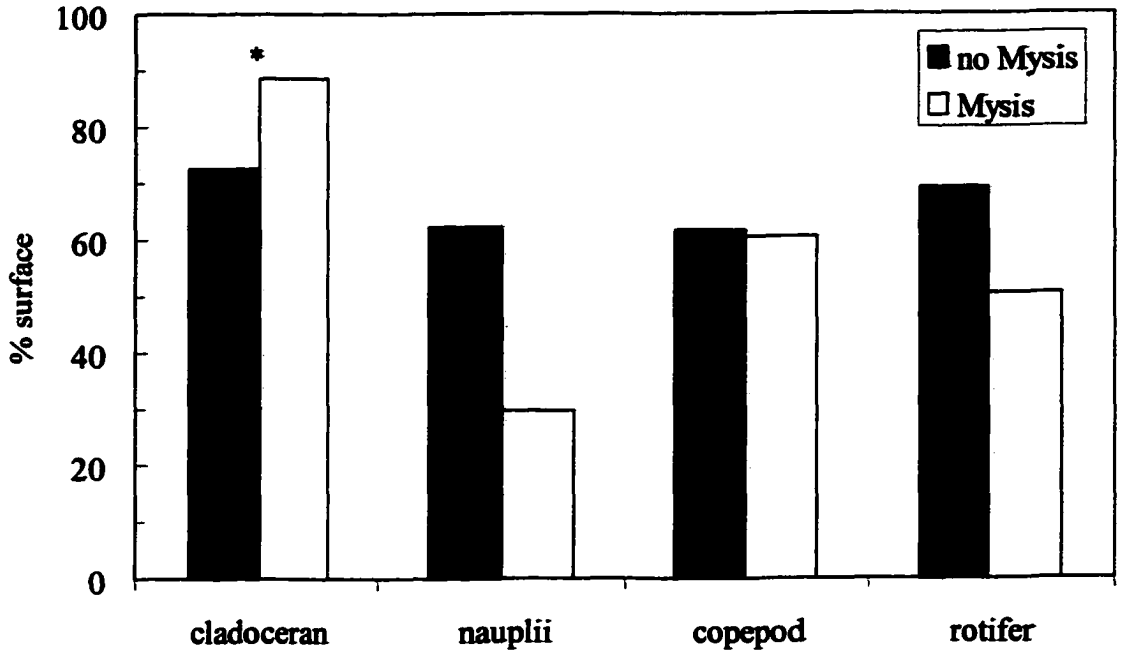


Figure 1. Average % surface densities for the zooplankton categories in lakes with and without Mysis (*significant difference at $p = 0.05$).

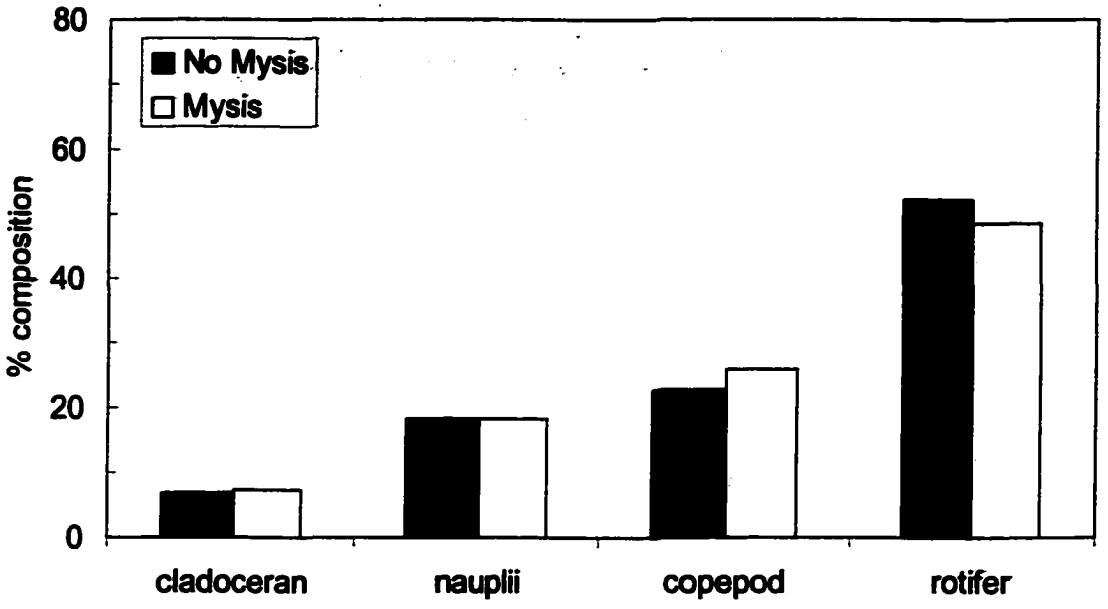


Figure 2. Overall zooplankton percent composition for combined surface and deep hauls calculated within lakes for each sampling event (n = 4) and subsequently averaged. No significant differences were observed between lakes with and without Mysis ($p \geq 0.10$ for all four comparisons).

CHAPTER 6

CHANGES IN LAKE TROUT GROWTH ASSOCIATED WITH MYSIS RELICTA ESTABLISHMENT: A RETROSPECTIVE ANALYSIS USING OTOLITHS

Craig P. Stafford, J. A. Stanford, F. Richard Hauer, and Edward B. Brothers

ABSTRACT

The establishment of *Mysis relicta* in Flathead Lake, Montana was followed by a dramatic increase in the lake trout (*Salvelinus namaycush*) population. Using otoliths, we compared lake trout growth pre and post *M. relicta*. Widths of annual increments 1 to 3 were similar, but generally declined in increments 4 to 10 post *M. relicta*. To calculate body size in the post *M. relicta* fish we used the relationship between body length and otolith radius. To retrospectively calculate body length for the pre *M. relicta* fish (for which we lacked body sizes), we developed a simple empirical correction to account for the conservative nature of otolith growth rate relative to body growth rate.

INTRODUCTION

Mysis relicta (Mysidacea: Mysidae) is an opossum shrimp native to northeastern and north central North America and northern Eurasia (Dadswell 1974). Fisheries managers introduced *M. relicta* in western North American and Scandinavia (Gregg 1976; Fürst 1981; Lasenby et al. 1986) largely based on the increased growth and sustained abundance of kokanee (*Oncorhynchus nerka*) after *M. relicta* addition to

Kootenay Lake, British Columbia (Sparrow et al. 1964; Northcote 1972). These results generally have not been duplicated elsewhere (Morgan et al. 1978; Beattie and Clancey 1991; Bowles et al. 1991) and appear to be related to the particular morphometry and nutrient enrichment of Kootenay Lake (Northcote 1972; Martin and Northcote 1991).

Mysis relicta remain on the bottom during the day and vertically migrate at night (Juday and Birge 1927) to feed, particularly on large cladocerans (Cooper and Goldman 1980; Spencer et al. 1999). The shrimp appear to shuttle a substantial quantity of macro zooplankton production from the pelagic zone to the profundal, and *M. relicta* introductions generally have decreased the abundance of pelagic planktivores while benefiting profundal fishes (Lasenby et al. 1986; Hammar 1988; Bowles et al. 1991; Langeland et al. 1991).

Between 1968-1976, *M. relicta* were introduced from Waterton Lake on the eastern side of the continental divide in Montana into 12 lakes on the western side. Populations were established in at least seven lakes (Crystal, Little Bitterroot, McGregor, Swan, Ashley, Whitefish and Holland), the latter four of which are upstream of Flathead Lake. By 1981, *M. relicta* had established in Flathead Lake via downstream drift (Spencer et al. 1991).

The establishment of *M. relicta* dramatically changed the food web of Flathead Lake. The density of cladocerans and the most abundant copepod declined post *M. relicta* (Spencer et al. 1991; Spencer et al. 1999). Lake-wide spring gill net monitoring captured 14 times more nonnative lake trout (*Salvelinus namaycush*) per sinking gill net from 1996 to 1998 than the average of 1981 and 1983 (due to the low number of lake trout captured in 1981 and 1983 this increase should be considered approximate).

Besides lake trout, only nonnative lake whitefish (*Coregonus clupeaformis*) showed an obvious increase in the nets. In contrast, the abundance of native bull trout (*Salvelinus confluentus*), cutthroat trout (*O. clarkii*) and nonnative kokanee decreased in the nets post *M. relicta* (Mark Deleray, Montana Department of Fish, Wildlife and Parks, Kalispell, Montana, personal communication). The bull trout and kokanee declines are also apparent from redd surveys (Beattie and Clancey 1991; Spencer et al. 1991; Rieman and Myers 1997).

The lake trout expansion in Flathead Lake is remarkable considering the relatively late maturity (> 4 years) of this species (Martin and Olver 1980), and the expanded lake trout population appears to be influencing other fishes in the lake. Lake trout have replaced bull trout as the dominant piscivore in Flathead Lake, and have been shown to compete heavily with bull trout in other systems (Donald and Alger 1993). Predation by the expanded lake trout population is impacting the abundance of other fishes in the lake, particularly kokanee (Carty et al. 1997).

The objective of this study was to examine growth changes in lake trout otoliths associated with *M. relicta* establishment in Flathead Lake using a sample collected post *M. relicta*. We were particularly interested in any insights the growth the patterns could provide into causes of the lake trout population expansion. To relate changes in otolith sizes to body lengths for the pre *M. relicta* fish (for which we lacked body sizes), we developed a simple empirical correction to account for the conservative nature of otolith growth rate relative to body growth rate. We independently validated this procedure using lake trout from nearby Lake McDonald.

METHODS

Flathead Lake (47 52 N, 114 04 W) and Lake McDonald (48 35 N, 113 55 W) are deep oligotrophic lakes of the Upper Columbia Basin in northwest Montana, with Lake McDonald being colder and less productive. These lakes have similar guilds of native and nonnative fishes, however Lake McDonald remains without *M. relicta*. More detailed information on Flathead Lake and its food web is provided in Spencer et al. (1991) and Stanford and Ellis (in press). Lake trout were captured in Flathead Lake using experimental gill nets ranging from 19 to 51 mm bar mesh from December 1986 to August 1995, and from Lake McDonald in 1991 and 1994. We used 151 fish from Flathead Lake and 14 from Lake McDonald for this analysis. Fish were weighed (g), total length (TL in mm) was determined, and sex was recorded for 117 of the Flathead Lake fish. Otoliths (sagittae) were removed from the fish and stored dry. Otoliths were embedded in epoxy and a transverse section was cut through the primordium. Sections were polished and annual increment widths (μm) were determined along an axis from the primordium to the dorsal margin. All otoliths were read by a single reader using a compound microscope with an image capture system. The first two or three increments were opaque and difficult to read. The incomplete increment was removed from calculations of average increment widths, but not for measurements of total otolith radii.

We compared otolith growth rate pre and post *M. relicta* (here after "pre" and "post", respectively) by comparing average otolith increment widths for the first 10 increments. Increments beginning in 1970 to 1984 were categorized as pre, while those after 1984 were considered post. We based this division on the first collection of *M.*

relicta in Flathead Lake in 1981 and the low densities from 1981 to 1984 (< 3 per m^2) (Spencer et al. 1991).

We used the average otolith radius at annulus and the TL versus otolith radius relationship to calculate TL at annulus pre and post after addressing several biases. We were concerned that the gill nets selectively captured fast growing young fish, so we compared the increment widths of young fish to the corresponding increment widths from the older fish to evaluate gill net selectivity. Several authors have reported that otolith growth rates are conservative relative to body growth rate (Templeman and Squires 1956; Reznick et al. 1989), so fast growing fish will have smaller otoliths at a given body size and vice versa. Thus any growth rate differences pre and post would be problematic for the calculated pre TL as the body size versus otolith size relationship was calculated from post fish. To resolve this issue we quantified the relationship between body and otolith growth rate and accounted for the effect on the calculated pre TL. Body growth rate was calculated as the residuals of the TL versus number of complete increments regression. Otolith growth rate was calculated as the residuals of the otolith radius versus TL regression. The relationship between body versus otolith growth rate was determined by regression of the corresponding residuals. The slope of this relationship was multiplied by the difference in the average post and pre otolith radius at each annulus where the otolith increment data indicated the growth patterns differed pre and post. This correction factor was added to the TL obtained by entering the pre otolith radius at annulus into the post TL versus otolith radius regression. To check this method we applied the analogous procedure to the Lake McDonald fish which we expected would have slower growth rates than the Flathead Lake fish.

We examined if *M. relicta* establishment corresponded to changes in the size of angler caught fish or diet. We examined trends in the total lengths of angler-caught lake trout using four year round creel surveys from 1961-1963 (Robbins 1966), 1981-1982 (Graham and Fredenberg 1982), 1992-1993 (Evarts et al. 1994) and 1998-1999 (Barry Hansen, Confederated Salish and Kootenai Tribes Tribal Fisheries Program, Pablo, Montana, personal communication). The most important (by weight) diet item for lake trout was described for 1980-1982 (Leathe and Graham 1982), 1986-1988, 1989 and 1996 (Montana Department of Fish, Wildlife and Parks, Dingell-Johnston reports), and 1998-1999 (Stafford et al in prep.). Stomachs were collected throughout the year for 1980-1982, 1986-1988, and 1996. Stomachs from 1989 were collected May to September, while the 1998-1999 collection was made from May to June and October to November 1998, and from March to June 1999. The 1980-1982, 1986-1988, and 1989 collections were primarily from larger fish (>500 mm total length), while those from 1996 and 1998-1999 contained similar proportions of smaller (<500 mm) and larger fish. For the 1996 and 1998-1999 collections, we documented the most important diet item using the larger fish only.

RESULTS

The Flathead Lake fish had 1 to 38 complete increments at capture and the Lake McDonald fish ranged from 2 to 15. T-tests revealed no significant increment width differences between Flathead Lake females and males for all sexed fish in increments 1 to 10 ($0.055 \leq p \leq 0.950$). Based on the similar otolith growth rates between the sexes, we pooled females and males for further analysis.

Our analysis focused on Flathead Lake fish that had 1 to 22 complete increments. We chose not to use the oldest fish because the sample was small. Fish less than 350 mm TL were captured infrequently and all fish with 1 to 3 complete increments were less than 350 mm TL (n = 31). We evaluated the selective capture of fast growing young fish by comparing the first 3 average increment widths for fish with 1 to 3 complete increments (all of which were post) to the corresponding post increment averages from fish with 4 to 22 complete increments. This analysis suggested the gill nets selectively captured fast growing fish with 1 or 2 complete increments (Table 1). Based on these findings and the small sample size of fish with 3 complete increments (n = 4), we removed fish with 1 to 3 complete increments from the pre versus post comparison of lake trout growth rate.

We compared the average widths of the first 10 increments pre and post with t-tests using fish captured with 4 to 22 complete increments. Mean increment widths were significantly smaller for all increments from 4 to 10 except for 5, while widths in increments 1 to 3 were similar (Fig. 1; Table 2). Otoliths from the Lake McDonald fish generally grew less than those from Flathead Lake, especially in increment 1 (Table 2).

Partial regressions revealed that the body size versus otolith relationship depended on growth rate. The relationship between body length versus number of complete increments was described for all Flathead Lake fish with 4 to 10 complete increments by: $TL = 226 + 45.0I$ ($n = 51$, $R^2 = 0.511$, $p < 0.001$). The relationship between otolith radius versus TL was: $R = 599 + 1.53TL$ ($n = 51$, $R^2 = 0.742$, $p < 0.001$). We used the residuals from these equations as indices of body and otolith growth rate, respectively, resulting in the relationship: $B = 0.000 - 0.364O$ ($n = 51$, $R^2 = 0.222$, $p < 0.001$) (Fig. 2). To determine if the body growth rate versus otolith growth rate relationship varied with fish

age we pooled the fish into categories based on number of complete increments: 4 to 7 (n = 22) and 8 to 10 (n = 29). We then created a multiple regression model of otolith growth rate, increment category, and the interaction to predict body growth rate. We found no significant interaction ($p = 0.853$), indicating that the body growth rate versus otolith growth rate relationship did not vary detectably between groups.

To calculate the pre and post body lengths we attempted to remove biases that may have been introduced by the conservative nature of otolith relative to body growth rate. We were concerned that the selective capture of fast growing young fish may have distorted the body size versus otolith size relationship. To reduce this bias we re-sampled fish with 1 to 3 complete increments, removing fish if any increment width was outside of one standard deviation of the corresponding increment mean based on the post fish with 4 to 22 complete increments. The new pooled mean for increment 1 in fish with 1 to 3 complete increments ($n = 18$) was 496 μm, increment two in fish with 2 to 3 complete increments ($n = 10$) was 218 μm, and increment three in fish with 3 complete increments ($n = 3$) was 168 μm. These values were similar to the corresponding average increment widths calculated from the older fish (Table 1). After removing the fast and slow growers, we calculated the overall body length versus otolith radius relationship using all fish with 1 to 22 complete increments by: $L = 0.0850R + 0.000341R^2 - 0.0000000917R^3$ ($n = 128$, $R^2 = .992$, $p < 0.001$) (Fig. 3). Because otolith growth rates were similar pre and post for the young fish, we used this equation to calculate TL at annuli 1 to 3 for both post and pre fish using the respective average otolith radius at annuulus. To generate the post body lengths for annuli 4 to 10 we calculated the body length versus otolith radius relationship using fish with 4 to 13 complete increments born in 1982 and later. Thus,

1985 (the first year considered post in this study) is the first date incorporating increment 4 (the first increment where otolith growth rate declined post). The TL versus otolith radius relationship for these fish was: $TL = -124 + 0.468R$ ($n = 58$, $R^2 = 0.773$, $p < 0.001$). This equation was used to generate the average post TL for annuli 4 to 10 using the post average otolith radius at annulus. We also used this equation with the average pre otolith radii at annulus to calculate uncorrected pre TLs for annuli 4 to 10. Because the pre growth rate was generally faster in increments 4 to 10 and the body size versus otolith size relationship depended on growth rate we applied our correction procedure (see methods) to calculate corrected pre body lengths at annuli 4 to 10. Accounting for the conservative nature of otolith growth rate relative to body growth rate increased the calculated pre body lengths, particularly for the older fish (Fig. 4).

We tested our correction procedure using the Lake McDonald fish. The body versus otolith size regression for the Lake McDonald fish with 4 to 15 complete increments was described by: $TL = -201 + 0.463R$ ($n = 12$, $R^2 = 0.791$, $p < 0.001$). We used this equation and the average radius at annulus to calculate TLs for annuli 4 to 10. The slow somatic growth rate of the Lake McDonald fish resulted in a body length versus otolith radius relationship that lay below the 95% confidence interval generated by the post fish with 4 to 13 complete increments. We reasoned that the body length difference between the post and Lake McDonald lines should be approximated by the Lake McDonald otolith radius at annulus minus the corresponding post radius multiplied by the slope of the post body growth rate versus otolith growth rate regression. Adding the correction increased the Lake McDonald body lengths so they were within the 95%

confidence interval of the post regression. The slower growth rate of Lake McDonald fish was responsible for the lack of overlap with the post fish for annuli 4 and 5 (Fig. 5).

The average length of angler-caught lake trout declined after the kokanee crash in 1987 (Fig. 6). The most important diet item for larger lake trout pre was kokanee which was replaced by lake whitefish after the kokanee crash (Table 3).

DISCUSSION

Contrary to our expectations, establishment of *M. relicta* in Flathead Lake did not correspond to detectable growth changes in the first three increments. *Mysis relicta* is an important forage item for immature lake trout, especially the younger fish (Eschmeyer 1956; Griest 1977). We have no diet data for very young lake trout (<200 mm) from Flathead Lake, but fish in the 200-500 mm range make extensive use of *M. relicta* (Stafford et al. in prep.) Positive relationships have been reported between *M. relicta* and growth of the young lake trout in Green Lake, Wisconsin (Hacker 1962, as cited by Martin and Olver 1980) and Twin Lakes Reservoir, Colorado (Griest 1977). Several factors may have contributed to our contradictory findings. *Mysis relicta* densities in Flathead Lake are generally low when compared to other systems (Spencer et al. 1991; Lasenby 1991). Further, the new forage provided by *M. relicta* may have been offset by increased intraspecific food competition associated with the dramatic lake trout population expansion. Any growth changes that did occur may have been obscured by size selective mortality, which can be particularly strong for early life stages (Miller et al. 1988; Hambright et al. 1991), or the opaque and difficult to read early otolith growth.

The reduced growth in the older lake trout post was most likely caused by increased intraspecific competition and changes in the forage fish community post. Prior to *M. relicta*, kokanee represented an abundant, preferred food source. Kokanee were the most important food item from the 1980-1982 collection. Kokanee remained the dominant food item in the 1986-1988 collection, even though kokanee spawners declined sharply in 1986 and were at low levels by 1987 (Spencer et al. 1991). After the kokanee crash, less preferred lake whitefish became the main food item, particularly for the larger lake trout. Bowles et al. (1991) also reported declines in adult lake trout growth (as measured by condition factor) associated with *M. relicta* establishment and kokanee declines in Priest Lake, Idaho.

The reduced adult growth post resulted in a pattern of increasing divergence in the lengths at annulus relative to the pre fish. The lower average length of angler caught fish primarily reflects the younger age composition of the post lake trout, and secondarily the reduced growth rate. Declines in the large lake trout fishery (> 640 mm) have also been reported in Lake Tahoe after *M. relicta* establishment and associated kokanee declines (Richards et al. 1991).

The reduced growth in the older fish post strongly suggests that increased growth, survival, or fecundity of adults was not responsible for the population expansion. However, despite the dramatic population increase, growth remained relatively constant in the first three increments suggesting the expansion arose from increased survivorship of young fish. The vertical migration of *M. relicta* transfers zooplankton production from the pelagic to the profundal. This food pump may have expanded the area suitable for immature lake trout to feed, facilitating the population expansion. The presumably

abundant spawning habitat in the rocky bottom of Flathead Lake and the large average size (i.e., high fecundity) of the lake trout pre appears to have primed the population to expand with the enhanced survival of the young fish.

The lake trout population expansion is a major component of the food web reconfiguration associated with *M. relicta* establishment in Flathead Lake. Although lake trout abundance probably was increasing slowly since their introduction in 1905 (Spencer et al. 1991), *M. relicta* appears to have facilitated the dramatic lake trout population expansion via improved survival of the young fish. It appears the expanded lake trout population is largely responsible for the kokanee declines in Flathead Lake (Carty et al. 1997), although the reduced populations of large zooplankton also need to be considered (Beattie and Clancey 1991; Spencer et al. 1999). Further, we strongly suspect the lake trout expansion is at least partially responsible for the post bull trout declines. In nearby Swan Lake, which until very recently lacked lake trout, bull trout have become more abundant after *M. relicta* introduction, although this has been confounded with changes in the angling regulations (Rieman and Myers 1997; Baxter et al. 1999). Nevertheless, it appears that *M. relicta* alone have not been detrimental to bull trout in this system. The contrasting bull trout population trends between Swan and Flathead Lakes illustrate how multiple species introductions can synergistically reconfigure food webs.

We found that otolith growth rate was conservative relative to body growth, as has been reported by others (Templeman and Squires 1956; Reznick et al. 1989; Lombarte and Lleonart 1993). This was apparent both within the Flathead Lake sample and in the Lake McDonald fish relative to the post Flathead Lake fish. Accounting for the conservative growth rate of otoliths relative to body growth rate increased the calculated

body lengths at annulus 4 and higher. Applying the analogous procedure to the slow growing Lake McDonald fish resulted in a body length versus otolith radius relationship similar to the Flathead Lake fish, supporting the validity of our approach. Further, the growth difference between the Lake McDonald fish and the Flathead Lake post fish was much greater than the pre and post growth difference. These results illustrate the need to account for the conservative nature of otolith growth rate relative to body growth rate when using otoliths to predict body size (see Morita and Matsuishi 2001). We found no evidence for differences in the body growth rate versus otolith growth rate relationship between the 4 to 7 or 8 to 10 complete increment groups. However, if the body growth rate versus otolith growth rates relationship varies with age we recommend calculating an age-specific correction.

Acknowledgment-Funding for otolith collection, preparation, and reading was provided by the Montana Department of Fish, Wildlife and Parks, the Salish-Kootenai Tribal Fisheries Program, and by the Bierman Professorship of the Flathead Lake Biological Station. We thank B. Hansen for lengths of angler caught lake trout and manuscript comments and M. Deleray for gill net data and manuscript comments. We acknowledge W. Beattie, M. Deleray, J. Fraley, D. Hanzel, L. Knotek, J. Tohtz, S. Rumsey and T. Weaver, whose Dingell-Johnston reports (F-46-R-1, F-46-R-3, and F-78-R-1-5) provided diet information.

REFERENCES

Baxter, C. V., C. A. Frissell, and F. R. Hauer. 1999. Geomorphology, logging roads, and the distribution of bull trout spawning in a forested river basin: implications for management and conservation. Transactions of the American Fisheries Society 128:854-867.

Beattie, W. D., and P. T. Clancey. 1991. Effects of *Mysis relicta* on the zooplankton community and kokanee population of Flathead Lake, Montana. American Fisheries Society Special Symposium 9:39-48.

Bowles, E. C., B. E. Rieman, G. R. Mauser, and D. H. Bennett. 1991. Effects of introductions of *Mysis relicta* on fisheries in northern Idaho. American Fisheries Society Symposium 9:65-74.

Carty, D., W. Fredenberg, L. Knotek, M. Deleray, and B. Hansen. 1997. Hungry Horse Dam fisheries mitigation: kokanee stocking and monitoring in Flathead Lake. Bonneville Power Administration, DOE/BP-60559-3, Portland, Oregon.

Cooper, S. D., and C. R. Goldman. 1980. Opossum shrimp (*Mysis relicta*) predation on zooplankton. Canadian Journal of Fisheries and Aquatic Sciences 37:909-919.

Dadswell, M.J. 1974. Distribution, ecology, and postglacial dispersal of certain crustaceans and fishes in eastern North America. National Museum of Natural Science, Publications in Zoology No. 11, Ottawa, Ontario.

Donald, D.B. and D. J. Alger. 1993. Geographic distribution, species displacement, and niche overlap for lake trout and bull trout in mountain lakes. Canadian Journal of Zoology 71:238-247.

Eschmeyer, P. H. 1956. The early life history of the lake trout in Lake Superior. Michigan Department of Conservation, Miscellaneous Publication No. 10, Ann Arbor, Michigan.

Evarts, L., B. Hansen, and J. DosSantos. 1994. Hungry horse fisheries mitigation plan: Flathead Lake angler survey. Bonneville Power Administration, DOE/BP-60479-1, Portland, Oregon.

Fürst, M. 1981. Results of introductions of new fish food organisms into Swedish lakes. Institute of Freshwater Research Drottningholm 59:33-47.

Graham, P., and W. Fredenberg. 1982. Flathead Lake fisherman census. Environmental Protection Agency, R008224014, Denver, Colorado.

Gregg, R. E. 1976 The ecology of *Mysis relicta* in Twin Lakes, Colorado. Bureau of Reclamation, REC-ERC-76-14, Denver, Colorado.

Griest, J. R. 1977. The lake trout of Twin Lakes, Colorado. Bureau of Reclamation, REC-ERC-77-4, Denver, Colorado.

Hacker, V. A. 1962. A summarization of life history information of the lake trout, *Salvelinus namaycush*, obtained in gill netting, finclipping and tagging studies at Green Lake, Wisconsin-1956-1961. Wisc. Conserv. Dept. Fish Manage. Div. Invest. Mem. 3:1-24.

Hambright, K. D., R. W. Drenner, S. R. McComas, and N. G. Hairston, Jr. 1991. Gape-limited piscivores, planktivore size refuges, and the trophic cascade hypothesis. Archiv für Hydrobiologie 121:389-404.

Hammar, J. 1988. Planktivorous whitefish and introduced *Mysis relicta*: ultimate competitors in the pelagic community. Finnish Fisheries Research 9:497-521.

Juday, C., and E. A. Birge. 1927. Pontoporeia and Mysis in Wisconsin lakes. Ecology 8:445-452.

Langeland, A., J. I. Koksvik, and J. Nydal. 1991. Impact of the introduction of *Mysis relicta* on the zooplankton and fish populations in a Norwegian lake. American Fisheries Society Symposium 9:98-114.

Lasenby, D. C. 1991. Comments on the roles of native and introduced *Mysis relicta* in aquatic ecosystems. American Fisheries Society Symposium 9:17-22.

Lasenby, D. C., T. G. Northcote, and M. Fürst. 1986. Theory, practice, and effects of *Mysis relicta* introductions to North American and Scandinavian lakes. Canadian Journal of Fisheries and Aquatic Sciences 43:1277-1284.

Leathe, S. A., and P. J. Graham. 1982. Flathead Lake fish food habits study. Environmental Protection Agency, R008224-01-4, Denver, Colorado.

Lombarte, A., and J. Lleonart. 1993. Otolith size changes related with body growth, habitat depth and temperature. Environmental Biology of Fishes 37:297-306.

Martin, N. V., and T. G. Northcote. 1991. Kootenay Lake: an inappropriate model for *Mysis relicta* introduction in north temperate lakes. American Fisheries Society Symposium 9:23-29.

Martin, N. V., and C. H. Olver. 1980. The lake charr, *Salvelinus namaycush*. Pages 205-277 *in* E. K. Balon, editor. Charrs Salmonid Fishes of the Genus *Salvelinus*. Dr. W. Junk Publishers, The Hague, The Netherlands.

Miller, T. J., L. B. Crowder, J. A. Rice, and E. A. Marschall. 1988. Larval size and recruitment mechanisms in fishes: towards a conceptual framework. Canadian Journal of Fisheries and Aquatic Sciences 45:1657-1670.

Morita, K. and T. Matsuishi. 2001. A new model of growth back-calculation incorporating age effect based on otoliths. Canadian Journal of Fisheries and Aquatic Sciences 58:1805-1811.

Morgan, M. D., S. T. Threlkeld, and C. R. Goldman. 1978. Impact of the introduction of kokanee and opossum shrimp on a subalpine lake. Journal of the Fisheries Research Board of Canada 35:1572-1579.

Northcote, T. G. 1972. Some effects of mysid introduction and nutrient enrichment on an oligotrophic lake and its salmonids. Internationale Vereinigun für theoretische und angewandte Limnologie Verhandlungen 18:1096-1106.

Reznick, D., E. Lindbeck, and H. Bryga. 1989. Slower growth results in larger otoliths: an experimental test with guppies (*Poecilia reticulata*). Canadian Journal of Fisheries and Aquatic Sciences 46:108-112.

Richards, R., C. Goldman, E. Byron, and C. Levitan. 1991. The mysids and lake trout of Lake Tahoe: a 25-year history of changes in the fertility, plankton, and fishery of an alpine lake. American Fisheries Society Symposium 9:30-38.

Rieman, B. E., and D. L. Myers. 1997. Use of redd counts to detect trends in bull trout (*Salvelinus confluentus*) populations. Conservation Biology 11:1015-1018.

Robbins, O. 1966. Flathead Lake (Montana) fishery investigations, 1961-1964. Fish and Wildlife Service, Technical Papers of the Bureau of Sport Fisheries and Wildlife 4, Washington, D.C.

Sparrow, R. A. H., P. A. Larkin, and R. A. Rutherglen. 1964. Successful introduction of *Mysis relicta* Lovén into Kootenay Lake, British Columbia. Journal of the Fisheries Research Board of Canada 21:1325-1327.

Spencer, C. N., B. R. McClelland, and J. A. Stanford. 1991. Shrimp stocking, salmon collapse, and eagle displacement. BioScience 41:14-21.

Spencer, C. N., D. S. Potter, R. T. Bukantis, and J. A. Stanford. 1999. Impact of predation by *Mysis relicta* on zooplankton in Flathead Lake, Montana, USA. Journal of Plankton Research 21:51-64.

Stanford, J. A. and B. K. Ellis. In press. Natural and cultural influences on ecosystems processes in the Flathead River Basin (Montana, British Columbia). Chapter 14 *in* J. S. Baron, editor. Rocky Mountain futures: an ecological perspective, Island Press, Covelo, California.

Stafford, C. P., B. Hansen, and J. A. Stanford. In prep. Mercury in the food web of Flathead Lake, Montana. Submitted to Northwest Science.

Templeman, W., and H. J. Squires. 1956. Relationship of otolith lengths and weights in the haddock *Helanogrammus aeglefinus* (L.) to the rate of growth of the fish. Journal of the Fisheries Research Board of Canada 13:467-487.

Table 1. Flathead Lake lake trout number of complete increments at capture and mean widths of annual increments one to three (μm) with sample size in parentheses to evaluate gill net selectivity. The last row denotes the 95% confidence interval of the post *M. relicta* fish with 4 to 22 complete increments.

	inc 1	inc 2	inc 3
1 complete	567 (13)		
2 complete	510 (14)	244 (14)	
3 complete	520 (4)	204 (4)	153 (4)
4 to 22 C.I.	462-528	204-233	155-171

Table 2. Average annual increment width for Lake McDonald, Flathead Lake pre *M. relicta*, Flathead Lake post *M. relicta*, sample sizes, and Flathead pre versus post *M. relicta* t-test p value for complete increments 1 to 10.

	1	2	3	4	5	6	7	8	9	10
McDonald μm	342	196	169	136	129	101	99	92	82	65
Flathead pre μm	487	214	173	152	142	133	119	106	98	91
Flathead post μm	495	218	163	138	131	117	107	94	79	70
McDonald n	12	12	12	12	11	9	8	8	6	5
Flathead pre n	87	75	62	52	46	36	28	23	19	18
Flathead post n	23	35	48	58	58	61	62	65	62	49
pre vs. post p	0.634	0.657	0.065	0.002	0.062	0.006	0.039	0.029	0.001	0.006

Table 3. Date captured, most abundant diet item (by mass) and sample size for lake trout (primarily > 500 mm TL) captured from Flathead Lake.

Date	Item	n
1980-1982	kokanee	15
1986-1988	kokanee	123
1989	lake whitefish	282
1996	lake whitefish	214
1998-1999	lake whitefish	177

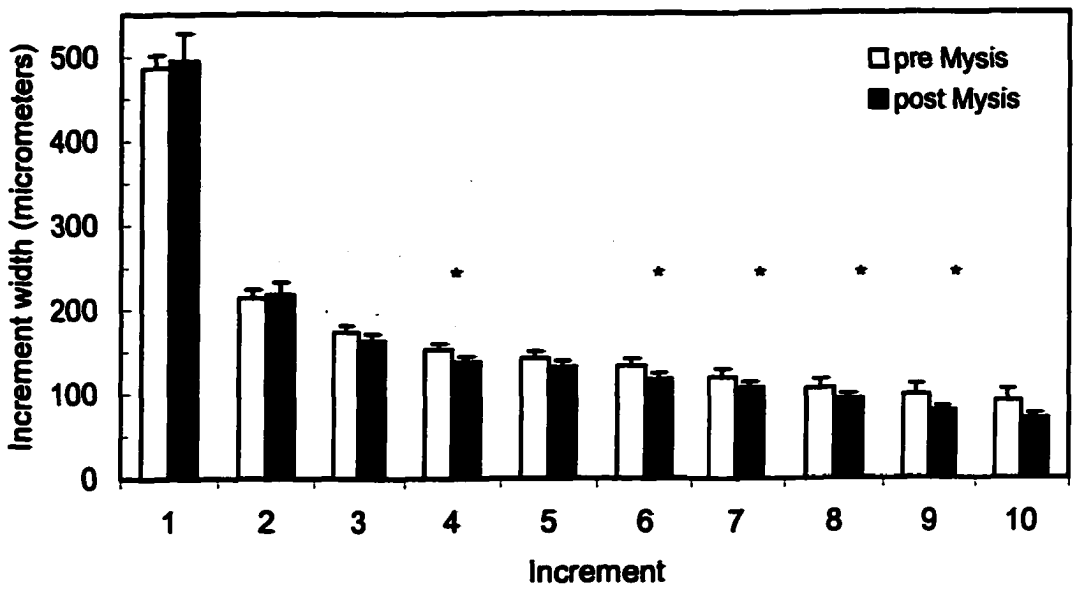


Figure 1. Annual increment widths of lake trout from Flathead Lake pre and post *M. relicta*. Error bars denote 95% confidence intervals. * denotes $p < 0.05$.

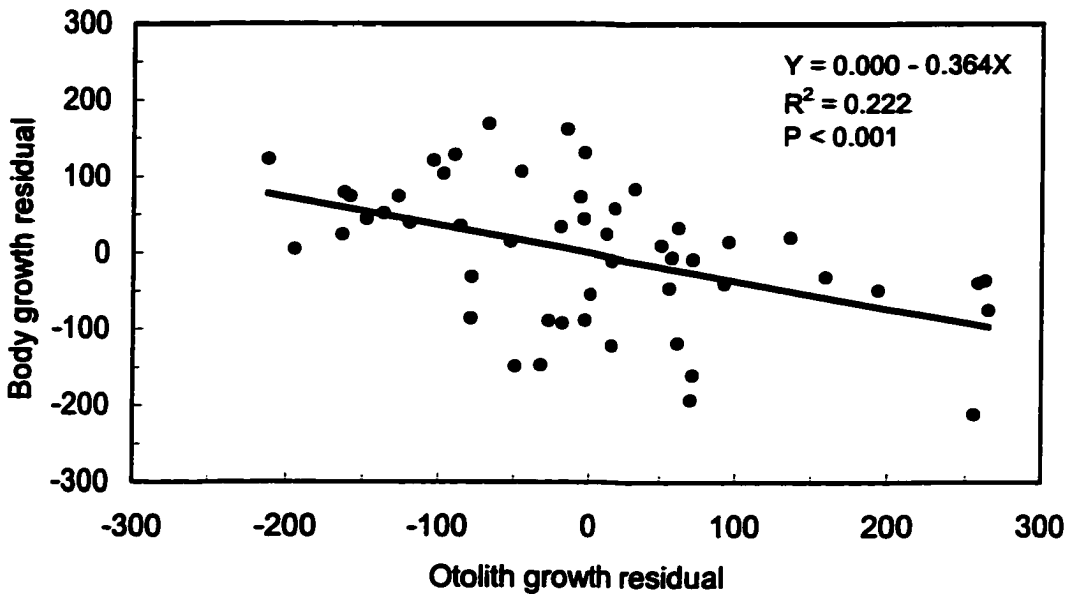


Figure 2. Body growth rate versus otolith growth for Flathead Lake fish with 4 to 10 complete increments.

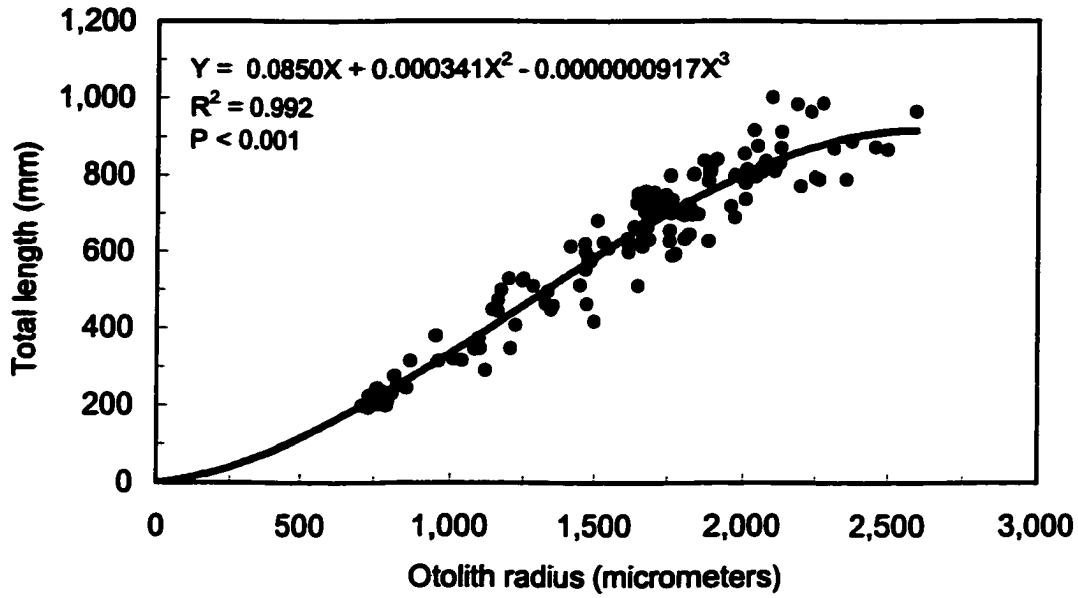


Figure 3. Total length versus otolith radius for Flathead Lake fish with 1 to 22 complete increments after removal of the fast and slow growing fish with 1 to 3 complete increments.

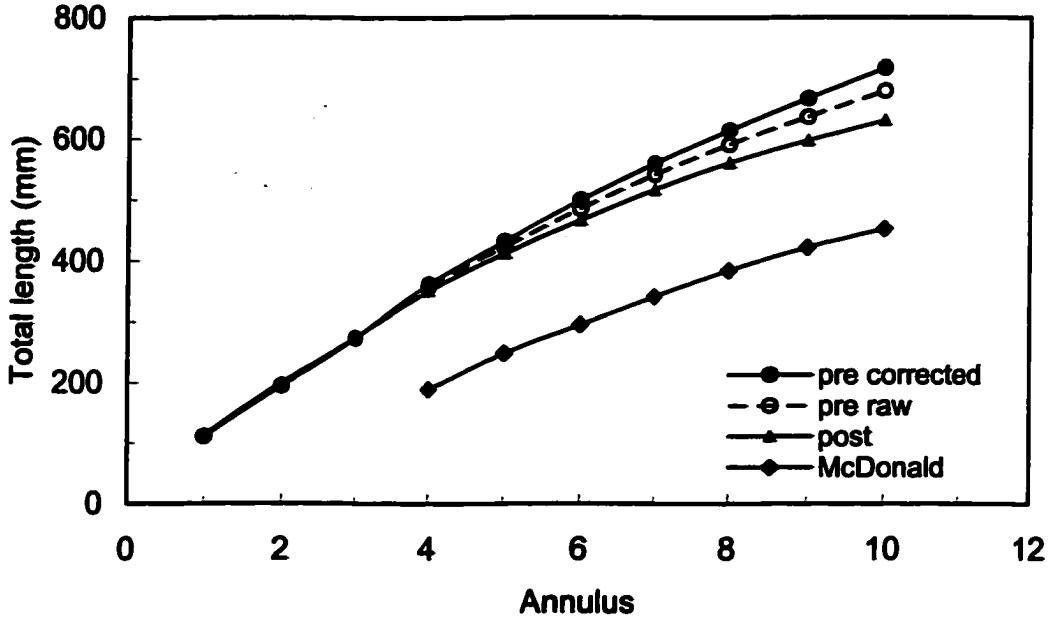


Figure 4. Total length at annulus for lake trout from Flathead Lake post *M. relicta* (post), pre *M. relicta* without accounting for the conservative nature of otolith growth rate relative to body growth rate (pre raw), pre *M. relicta* after accounting for the conservative nature of otolith growth rate relative to body growth rate (pre corrected), and Lake McDonald (McDonald).

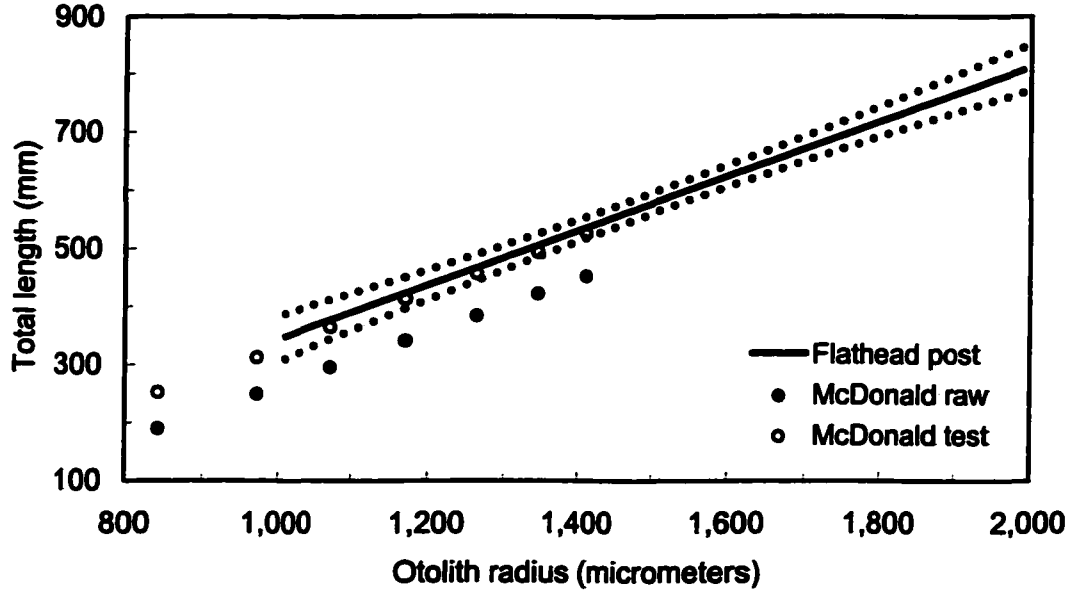


Figure 5. Total length versus otolith radius for Flathead Lake post *M. relicta* lake trout with 95% confidence interval (Flathead post), Lake McDonald lengths at annulus 4 to 10 (McDonald raw), and calculated Lake McDonald lengths to test the correction procedure (McDonald test).

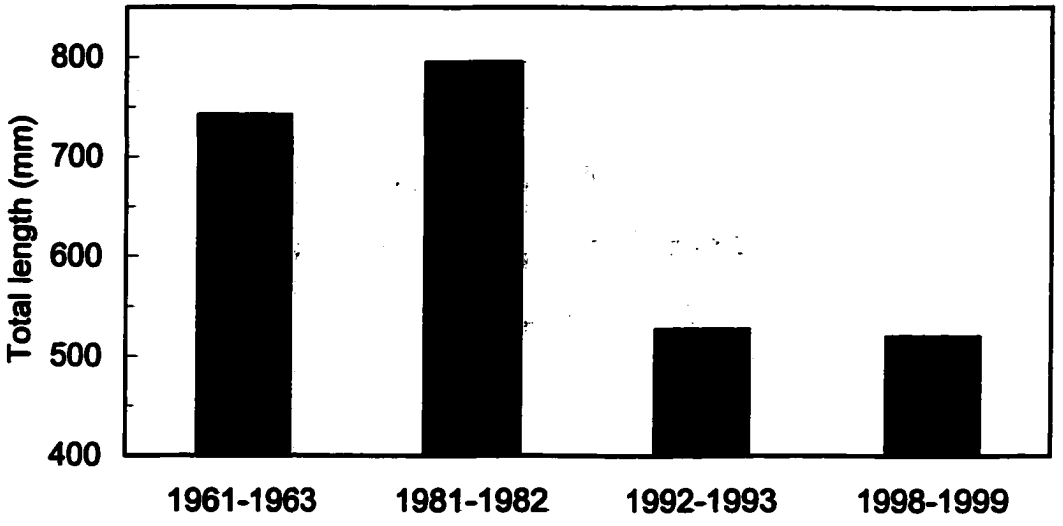


Figure 6. Average total length of angler caught lake trout from Flathead Lake by date. *Mysis relicta* were first detected in Flathead Lake in 1981 and became abundant in 1985.